The Impact of Estradiol and Stress on Spatial Memory

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Spatial memory is a critical cognitive function that involves for navigating, interpreting, and representing environments, planning a route, remembering the location of an object or event, and understanding spatial configurations. The three studies presented are original investigations on how estradiol, anxiety, and stress influence hippocampal-dependent spatial memory. The first study assessed the impact of estradiol level on spatial memory in young adult women. Salivary assays were obtained to determine the exact estradiol level. Women at the high and low estradiol phases of their natural menstrual cycle were compared to each other and to women on oral contraceptives (OCs). Behavioral performance on a virtual version of the Morris water task, a virtual version of the radial arm maze, and a mental rotation task was examined. Results indicated that higher levels of circulating estradiol were shown to be beneficial in comparison to lower levels for memory retention in spatial navigation across a 24-hour period. The second study examined how general anxiety levels impacts learning and memory. Performance on spatial and verbal memory tasks in men, women in the ovulatory phase their cycle, women in the non-ovulatory phase of their cycle, and women on OCs was measured. Behavioral performance on the virtual Morris water task, a declarative verbal memory task, and a verbal fluency task was assessed. Results indicated that general anxiety had no impact on spatial or verbal memory. Finally, the third study explored how an acute induced stressor affected spatial memory in men, women in the ovulatory phase their cycle, women in the non-ovulatory phase of their cycle, and women on OCs. Stress was induced via a virtual version of the Trier social stress task. Behavior was measured on the Morris water task, an object location task, a spatial working memory task, and a mental rotation task. Both men and women that underwent induction of stress had protected memory retention in spatial navigation compared to control participants. Induced stress also impaired performance on an object location task in men but had no impact in women. Stress had no impact on spatial working memory and mental rotation ability. Overall, the findings from all three studies suggest that estradiol level impacts spatial memory retention, anxiety does not affect spatial memory, and induced stress does influence some spatial tasks, dependent on sex.
The Impact of Estradiol and Stress on Spatial Memory

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The Impact of Estradiol and Stress on Spatial Memory

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I. General Introduction

1.1 Memory Systems

How do we remember where we left our keys if they are lost? Once we find them, how do we know how to navigate home without a GPS? Once at home, how do we recognize the living room is our own and not someone else’s? The human brain is designed to process intricate memories that allow us to function on a daily basis. Our memory systems allow us to find lost items when other relevant information is remembered, remember routes and locations, and recognize whether an environment is familiar or strange amongst myriad other types of memories. There are different types of memory that require distinct processing and affect behavior in varying manners.

Scientists had a major breakthrough in understanding memories in the 1950s with the introduction to a patient, famously known as HM. Prior to this, many neuroscientists believed that memories were distributed across the cortex (Jeffery, 2017; Squire, 2009b). However, with the discovery of memory deficits in patient HM, a whole new era of memory research began. In order to cease very severe seizures, HM had parts of both temporal lobes removed (Scoville & Milner, 1957). Neuroimaging of HM’s brain showed that the bilateral resection of the medial temporal lobe (MTL) resulted in partial removal of the hippocampus and surrounding areas, such as the amygdala, parahippocampal gyrus, entorhinal cortex, and portions of the perirhinal cortex (Corkin et al., 1997).

As a result of the surgery, HM was not able to form some types of new memories, forgot daily events, and had partial retrograde amnesia (Milner, 1962; Milner et al., 1968). However, his intellect and perceptual functions were intact, and he could hold on to temporary information; for example, he was able to hold a conversation and remember a digit span with constant rehearsal, without interruptions, for as long as 15 minutes (Milner, 1962; Milner et al., 1968).
This led researchers to make distinctions between immediate/short-term memory and long-term memory. With continued observations of HM, researchers found that not all types of long-term memories were hindered. Milner (1962) found that HM was able to improve on a mirror-tracing task that required remembering visual and motor information from day to day. HM was asked to draw a line in between the outlines of two star shapes based on what he could see in a mirror reflection. Even though HM had no recollection of previously completing the task, his performance improved daily. He had decreases in both time and errors across three days (Milner, 1962). Follow up studies showed that although HM’s performance was inferior compared to healthy controls, he was still able to consistently improve over several days of testing (Corkin, 1968). Additional work demonstrated that HM was able to retain that information for up to a year after initial learning (Gabrieli et al., 1993). Thus, researchers realized that not all types of new long-term memories are processed in the areas resected from the MTL (Cohen & Squire, 1980; Corkin, 2002). The findings from the work done with HM suggest that acquisition and retention of some visual and motor learning rely on regions outside of the MTL and that there are distinctions in how different types of memories are learned.

Many researchers distinguish two types of long-term memories: declarative and non-declarative. Declarative memory refers to conscious knowledge or recollection of facts and events (Bird & Burgess, 2008; Cohen & Squire, 1980; Eichenbaum, 1999; Jeffery, 2017; Squire, 2009a; Squire, 2009b; Squire & Zola 1996). More specifically, declarative memory refers to general relational processing that can occur across different species (Cohen & Eichenbaum, 1993; Squire & Zola-Morgan, 1991) including but not limited to monkeys and rodents (Burwell et al., 1995; Mishkin, 1978; Suzuki & Amaral, 1994). Non-declarative memory refers to memory for learning skills, habits, conditioning, emotions, priming, and procedures (Bird & Burgess,
Based on research with HM, it was evident that only his declarative memory was impaired by the MTL resection and that his non-declarative memory was spared (Corkin, 2002; Jeffery, 2017; Squire, 2009a; Squire, 2009b).

1.2 The Hippocampus and Memory Processing

The hippocampus, housed in the medial temporal lobe, plays a large role in consolidating most types of memories (Anderson et al., 2007; Bird & Burgess, 2008; Squire, 2009a). Surrounding regions such as the entorhinal cortex, parahippocampal cortex, and perirhinal cortex also aid hippocampal functioning (Anderson et al., 2007; Bird & Burgess, 2008). The hippocampus has connections to various cortical structures including the anterior thalamic nuclei, mammillary bodies, and basal forebrain (Anderson et al., 2007; Bird & Burgess, 2008). The three major hippocampal subdivisions include the dentate gyrus (DG), Cornu Ammonis area 1 (CA1), and Cornu Ammonis area 3 (CA3; Anderson et al., 2007; Duvernoy, 2005). The major excitatory neurons in CA1 and CA3 are pyramidal neurons, whereas in the DG, the major excitatory neurons are granule cells. All three major regions receive inputs from the entorhinal cortex (EC) and have different projections to various regions of the cortex. The EC is a key component in connecting the hippocampus to other areas of the cortex (Anderson et al., 2007; Duvernoy, 2005). While there are additional regions within the hippocampus such as Cornu Ammonis area 2 (CA2) and the subiculum, most researchers focus on the DG, CA1, and CA3 (Thompson et al., 2008).

The hippocampus is subject to neuroplasticity in that structural changes occur due to learning, ovarian steroid hormones, stress, and neurological damage (Anderson et al., 2007; Bruel-Jungerman et al., 2007). One type of plasticity is long-term potentiation (LTP) that occurs due to learning. LTP strengthens synaptic connections as a result of experience and is theorized
to be a model for memory (Bliss & Lomo, 1973; Bliss 1979). The induction of LTP in the hippocampus further explains its’ role in memory formation and consolidation (Bliss & Collingridge, 1993; Frey et al., 1993; Malenka, 1994). The dentate gyrus region of the hippocampal formation is one of the few regions in the brain that undergoes neurogenesis throughout adulthood (Anderson et al., 2007; Bruel-Jungerman et al., 2007; Epp et al., 2007; Gould et al., 1999). New neurons are formed from progenitor cells housed in the DG. Neurogenesis typically occurs as a result of learning and memory but can also occur based on changes in hormones and has been seen to occur in the hippocampus across many species (Anderson et al., 2007; Bruel-Jungerman et al., 2007). Overall, it is clear that the hippocampus is susceptible to structural changes and that LTP occurs as a result of learning and memory.

There are many different theories about how the hippocampus is involved in memory processing. One of the earliest theories is known as the cognitive-map theory. This theory stipulates that the hippocampus represents locations of environments in a spatial or cognitive map (Nadel & MacDonald, 1980; O’Keefe & Dostrovsky, 1971; O’Keefe & Nadel, 1978). This theory was predicated on the discovery of place cells. Place cells were first discovered when researchers found that specific hippocampal neurons had increased firing when a rat was in a particular location relative to its environment (O’Keefe & Dostrovsky, 1971). Others have shown that even during free movement within a space, place cell activity in rats is specific to spatial contexts (Wilson & McNaughton, 1993). However, studies have also shown that when rats run on a linear track, place cells remap based on the direction of movement (Gothard et al., 1996; Redish et al., 2000). Remapping occurs when a familiar environment is altered and the place cell firing pattern changes (Bird & Burgess, 2008). Additionally, others have shown that place cell firing changes based on the rat’s future goal of intended movement (Ainge et al., 2007;
Ferbinteanu & Shapiro, 2003; Lee et al., 2006; Wood, et al., 2000). Specifically, when a rat navigates toward a goal location on a T-maze, the pattern of firing in place cells differs depending on whether the goal is on the right or left arm (Lee et al., 2006). Place cells have also been seen in humans (Ekstrom et al., 2003) and monkeys (Ono et al., 1991). In one study with humans, researchers found that place cells changed firing patterns based on intended goal location within a virtual reality environment (Ekstrom et al., 2003). Furthermore, many researchers agree that the hippocampus not only codes for space, but for time as well (Eichenbaum, 2014; Eichenbaum & Cohen, 2014; Ekstron & Ranganath, 2018; Konkel et al., 2008; Konkel & Cohen, 2009). Thus, although the cognitive map theory is not able to account for all hippocampal functioning, it is evident that the hippocampus is involved in processing spatial information.

Another theory of hippocampal processing is the declarative theory. This theory states that the hippocampus, along with other regions in the MTL, is essential for all types of declarative memories including episodic and semantic memory (Eichenbaum, 1999; Tulving, 1972). Once memory is formed and consolidated, after some time, the older memories would be stored in areas of the cortex and thus would be unaffected by MTL damage (Eichenbaum, 1999; 2000; 2004). In contrast to this theory, the multiple-trace theory suggests that the hippocampus is involved in episodic memories across life-time (Nadel & Moscovitch 1997; Nadel et al., 2000). According to this theory, even if consolidated memories are stored in the cortex, every time the memory is retrieved, it becomes vulnerable to change and thus is impacted by hippocampal functioning when the memory is to be stored once again (Nadel & Moscovitch, 1997; Nadel et al., 2000). Finally, another theory supports the declarative theory but states that the hippocampal role in memory is much more flexible. According to the relational theory, the hippocampus
processes relations between elements of an event that can be retrieved in novel situations to understand new events (Cohen & Eichenbaum, 1993; Cohen et al., 1997; Eichenbaum & Cohen, 2004). In this theory, the cognitive-map theory could be considered one type of relational processing, specifically with spatial elements (Cohen & Eichenbaum, 1993; Eichenbaum & Cohen, 2004; Konkel & Cohen, 2009).

1.3 Spatial Memory

Spatial memory is a cognitive mechanism that is critical for everyday life. Strong spatial skills allow for proper navigation, interpretation, and representation of environments. In memory research, there are numerous spatial tasks that tap into hippocampal-dependent spatial ability. Some tasks require the use of egocentric information in which we understand objects in space in an environment relative to our own body (O’Keefe & Nadel, 1978). Other tasks require the use of allocentric information in which we understand spatial representations based on the relationships between objects in an environment, independent of our location (O’Keefe, 1991; O’Keefe & Nadel, 1978).

Spatial tasks were designed to test various types of spatial memory including navigation, object location, mental rotation ability, and much more. One of the initial spatial navigation tasks created was the radial arm maze. In this task, rats start at a central point from which they can navigate down the extending arms to receive a food reward in certain arms (Olton & Papas, 1979; Olton & Samuelson, 1976; Olton et al., 1978). This task, which does involve spatial memory, also tests for working and reference memory. It tests for working memory in that animals were to hold on to the information of which arms had been entered within a trial. The task tests for reference memory in that animals were to remember which arms were baited with a reward and which were not, in order to save time and energy from entering into a non-rewarded
Researchers found that damage to areas that are connected to the hippocampus, such as the entorhinal cortex, fornix, and septum, leads to impaired performance on this task in rats (Olton & Papas, 1979; Olton et al., 1978). Another spatial navigation task was also designed to test spatial memory in rodents, specifically with the intent of eliminating a possibility of using non-spatial methods to complete it. The Morris water maze, originally created by Richard Morris, is designed to test a rodent’s ability to use extra-maze cues in an environment to navigate to a platform submerged in water (Morris, 1981). Researchers found that hippocampal and subiculum lesions result in profound deficits on this task (Morris et al., 1982; Morris et al., 1990). Studies have also shown that damage to the hippocampus and surrounding areas leads to dysfunction in spatial navigation in humans (Astur et al., 2002; Bohbot et al., 1998; Burgess et al., 2002).

1.4 Sex Differences in the Hippocampus

Sex differences refer to the biological distinctions between males and females that are determined during sexual conception (Arnold, 2004). Separate from sex differences, gender differences are influenced by environmental, societal, or cultural factors and refer to one’s self-representation (Cosgrove et al., 2007; Gillies & McArthur, 2010). Areas in the brain that exhibit sex differences are known as sexually dimorphic regions. Many scientists have investigated how and why those differences occur. For example, it was observed that adolescent women typically hit their peak gray matter volumes a year earlier than adolescent men; however, men have a steeper increase in white matter (Lange et al. 1997). The developing brain during pubertal years is vulnerable to the effects of gonadal steroid hormones and establishes many neural connections that last throughout adulthood (Cooke & Woolley, 2004; Gillies & McArthur, 2010; McEwen, 2007; Neufang et al., 2008). Additionally, Lange and colleagues (1997) found that the overall amygdala volume typically increases more throughout adolescence in men than women, whereas
hippocampal and striatal volumes typically increase more in women compared to men. Differences in brain development between the sexes may be related to differences in behavior, learning, and memory.

Prior research has established general patterns of sex differences in the brain across different animals. Some researchers have focused on differences in structure (Cahill, 2006; Choleris et al., 2018; Conejo et al., 2003; Cosgrove et al., 2007; Roof, 1993; Thompson et al., 2008), whereas others have examined consequences in function (Isgor & Sengelaub, 1998; Wei et al., 2016). The hippocampus has been considered sexually dimorphic in both structure and function (Cahill, 2006). Some studies have shown that men typically have larger hippocampi compared to women when adjusting for overall brain volume (Cahill, 2006; Cosgrove et al., 2007). Furthermore, others have shown greater sexual dimorphism in brain areas that showed greater levels of sex steroid receptors during pubertal periods of development (Goldstein et al., 2001). This suggests that structural sex differences may be at least partially mediated by sex steroids (Andreano & Cahill, 2009). Furthermore, a recent meta-analysis discovered that although men do have larger hippocampi, when adjusting for total brain or intracranial volume, the sex difference disappears and is misrepresented in some research (Tan et al., 2016). These researchers found that when uncorrected hippocampi were compared between sexes, men had a larger hippocampus compared to women by 6 to 7%. However, when overall total brain volume and intracranial volume were corrected for, the difference was reduced to 0.6% which was not significantly different between sexes (Tan et al., 2016).

In addition to sex differences in overall hippocampal size, further distinctions exist within the regions of the hippocampus. For example, male rats typically have a larger CA1 region and more pyramidal cells in the region compared to female rats (Madeira & Lieberman, 1995;
Madeira et al., 1992). These researchers found that the pyramidal cell layer is 16% smaller and the total number of CA1 neurons is 20% lower in females compared to males (Madeira et al., 1992). However, it is important to note that given the time period in which these studies were conducted, it was unlikely that the overall brain volume was accounted for, especially since it was not reported.

Sex differences in the CA1 and CA3 regions of rats and humans also occur in the size of pyramidal neuron cell bodies, number of primary dendrites, and degree of dendritic branching, which has been shown to be associated with behavioral performance on hippocampal-dependent spatial tasks (Isgor & Sengelaub, 1998). Isgor and Sengelaub (1998) investigated how prenatal exposure to testosterone propionate (TP), dihydrotestosterone propionate (DHTP), or estradiol affected behavior in rats on spatial navigation tasks. Female rats without hormonal treatment had significantly lower CA1 pyramidal cell field volumes. Females that received TP had a 26% larger average volume and females that received estradiol had a 22% larger average volume compared to females without treatment. Similar results were seen for CA3, except that females without treatment and females treated with estradiol did not differ from each other but each had smaller volumes compared to females that received either TP (20%) or DHTP (13%). The same pattern between groups was seen in analyses of soma size for CA1 and CA3 as seen in analyses of cell field volume (Isgor & Sengelaub, 1998). Behavioral results indicated that non-treated control females had impaired performance than males on escape latency to a submerged platform and percent of time spent in the correct quadrant of the pool during a probe trial. Females prenatally treated with TP, DHTP, or estradiol performed comparably with males on both behavioral measures (Isgor & Sengelaub, 1998). Overall, all three hormones were able to reduce a deficit often seen in control females without prenatal hormonal treatment. While testosterone
was most affective in improving behavioral performance and generating benefits in CA1 volume size, estradiol was shown to be an important hormone that yielded similar results (Isgor & Sengelaub, 1998).

Additionally, there are differences in the volume of the anterior versus the posterior hippocampus as well (Persson et al., 2014). In one study, researchers examined the size of the anterior and posterior hippocampi in men and women aged 20-35 years old. Results indicated that men had larger total intracranial volumes than women. However, in accounting for this difference, the researchers found that women had larger posterior hippocampi compared to men by 4.3%. Anterior hippocampi were comparable in men and women. Behaviorally, men performed better than women on a virtual Morris water maze and on another spatial task in which participants navigated through a maze and had to indicate the location of the start point (Persson et al., 2014). Another study replicated the result that young adult women had larger posterior hippocampi (Wei et al., 2016). They also found that young adult men had larger anterior hippocampi and found that this difference had a weak positive correlation ($r = .19$) with behavioral performance on a mental rotation task for both sexes (Wei et al., 2016).

### 1.5 Sex Differences in Spatial Memory

Sex differences have been observed in multiple spatial modalities including general spatial memory (Dabbs Jr. et al., 1998; Driscoll et al., 2005; Epting & Overman, 1998; Lewin et al., 2001; Maccoby & Jacklin, 1978; McGivern et al., 1997), spatial rotation (Galea & Kimura, 1993; Linn & Petersen, 1985; Moffat et al., 1998; Silverman et al., 2007; Vandenberg & Kuse, 1978), spatial navigation (Astur et al., 1998; Dabbs Jr. et al., 1998; Galea & Kimura, 1993; Moffat et al., 1998; Postma et al., 2004; Sandstrom et al., 1998; Silverman et al., 2000), and object location (Choi & Silverman, 1996; James & Kimura, 1997; McBurney et al., 1997;
Specifically, it has been shown that males typically outperform females on spatial navigation tasks in human research (Astur et al., 1998; Galea & Kimura, 1993; Moffat et al., 1998; Postma et al., 2004; Sandstrom et al., 1998). In contrast females have been shown to have enhanced performance in object location compared to males (Choi & Silverman, 1996; Honda & Nihei, 2009; James & Kimura, 1997; McBurney et al., 1997; McGivern et al., 1997; Levy et al., 2005; Silverman et al., 2007; Spiers et al., 2008). Many theories approach explanations for why and how sex differences occur in spatial behaviors. Three of these theories are discussed below: hunter-gatherer theory, differences in spatial processing, and differences prevalent during childhood.

1.5.1 Hunter-Gatherer Theory: The hunter-gatherer theory was introduced by Silverman and Eals in 1992. It postulates that sex differences occur in spatial abilities due to the evolutionary division of labor between sexes in early Homo sapiens. Specifically, males are thought to have certain characteristics due to their original role as ‘hunters’ and females were thought to have adapted other abilities based on their role as ‘gatherers’. Given the major role divisions, these researchers identified key features that allow each sex to have evolutionary adaptations and advantages based on their specific needs (Eals & Silverman, 1994; Silverman et al., 2007; Silverman et al., 2000; Silverman & Eals, 1992).

As part of this theory, Silverman and Eals (1992) state that male hunters need special skills and abilities in order to successfully hunt and provide food for their families. Tracking animals across familiar and unfamiliar territories and accurately placing targets to stun or kill them requires spatial skills related to the ability to orient oneself in relation to objects and places. These skills would be needed in distances that were visible or conceptualized, requiring the use of mental transformations. The researchers state that many of these aspects can be examined in
modern cognitive mechanisms such as mental rotations, map reading, and maze learning, all of which typically show a male advantage (Eals & Silverman, 1994; Silverman & Eals, 1992). In contrast, in order to successfully gather and forage for food, females would need to excel in locating food sources within a spatial array of vegetation. Furthermore, it would be ideal for them to be able to relocate these sources during different seasons. Thus, females would have great recognition and recollection of spatial configurations of objects, contents of object arrays, and spatial relationships between objects. Additionally, gatherers and foragers likely pick up on information from surrounding areas without intent or purpose. This incidental memory for objects and their locations would be helpful in understanding the areas around one’s shelter (Eals & Silverman, 1994; Silverman & Eals, 1992).

Given the distinctions in spatial skills between sexes, researchers conducted many experiments in which they tested spatial memory. Across a series of experiments, Silverman and Eals (1992) examined how men and women performed in learning content and spatial configurations of object arrays. In some experiments object arrays were presented in drawings, in others they were presented in real life offices. Regardless of how the object arrays were presented, women typically had better performance indicated by better object and location memory than men. Women were much more likely to remember which objects were presented in original arrays after distractor objects were added and the original locations of those objects even after some were moved compared to men (Silverman & Eals, 1992). These results were consistent with the hunter-gatherer theory of sex specific spatial memory considering women displayed superior performance on object, location, and incidental memory.

In follow up experiments, Eals and Silverman (1994) attempted to test the same theory while controlling for any female advantage with verbal skills. The researchers attempted to
negate any confound that may result from women typically having a greater ability to verbally express themselves. In doing so, they replicated the original studies and controlled for verbal explanations of objects and locations. Results indicated that females once again performed better for location memory than men. However, there were differences for the type of object memory. For example, in certain circumstances, participants were instructed to try and learn the objects in a room and remember the locations, known as directed learning. In another condition, participants were unaware that their time spent in the room was related to the study, thus any information learned during that time was considered incidental. Women excelled in incidental learning while men performed better on directed learning. The researchers state that incidental learning can be regarded as the most prominent type of learning that occurs in natural foraging situations. According to the hunter-gatherer theory, females have strong spatial abilities in understanding objects and locations around them even when not intentional. Furthermore, these experiments concluded that the female advantage on these tasks was independent of verbal skills (Eals & Silverman, 1994).

Through the original set of experiments, the spatial characteristics related to females as gatherers were further developed. Silverman and colleagues (2000) then attempted to identify the spatial skills that aided males in hunting. They examined how males and females differed in wayfinding, a process in which people navigate through unfamiliar territories with the objective of returning to the original point. According to the hunter-gatherer theory, males would require excellent wayfinding skills in order to track and hunt animals through unknown terrain. They would also need to find the most direct route home. The theory also stipulates that these spatial abilities correspond to the ability to mentally rotate an environment and understand all possible configurations. Thus, the researchers examined how wayfinding abilities related to mental
rotation performance. Participants were guided through the woods on a specific path and were asked to navigate back to the start once the path was completed. Results indicated the males performed significantly better on both wayfinding and mental rotation compared to women. Furthermore, they found that performance on the two tasks was positively correlated (Silverman et al., 2000).

Overall, the research reviewed presents a possible explanation for sex differences in spatial behavior. However, the hunter-gatherer theory does not account for differences in brain processing, hormonal influences, and experience with spatial abilities. Furthermore, the theory cannot explain why or how rodent models would display sex differences on spatial tasks given no division of roles in these animals.

1.5.2 Sex Differences in Spatial Processing: Given that there are different types of information available in spatial navigation events, researchers have attempted to understand which features are more salient to each sex. Studies with non-human models were helpful in first identifying sex differences in spatial processing. Williams and colleagues (1990) tested rats with hormonal manipulations on a radial arm maze. Male rats were either castrated neonatally (MNC) or were controls (MC) while female rats were either neonatally treated with estradiol (FNE) or were controls (FC). On a standard radial arm maze, results indicated that the MC and FNE groups performed the best, indicated by fewer errors, compared to the other two groups. In a second experiment, rats completed another version of the task in which geometric and landmark information was modified. Results indicated that the MC and FNE groups had impaired performance by manipulation of geometric information but were unaffected by landmark alteration. The FC and MNC groups had no change in behavioral performance based on manipulations of geometric or landmark information (Williams et al., 1990). The findings of this
study implicate the differences that occur between sexes in spatial behavior. Furthermore, the implications of this study emphasize the important role of gonadal hormones in spatial memory performance. The researchers postulate that these sex differences and the influence of hormones are likely related to processing in the hippocampus (Williams & Meck, 1991).

Given the findings from rodents, many researchers have investigated sex differences in spatial processing in humans. Early studies tested how men and women perform on map learning and strategies there were implemented (Galea & Kimura, 1993). Participants had to learn novel routes on a map based on specific cues. Results indicated that men performed better than females on the overall task with fewer errors. Men also had better memory for the geometric properties of the map. In contrast, women had better memory for landmarks on the map, even those that were unrelated to the learned route (Galea & Kimura, 1993). The findings of this study further imply sex differences in processing specific features of spatial navigation.

Moffat and colleagues (1998) aimed to examine how men and women performed on a 3D virtual maze that modeled more realistic navigation. The task was designed specifically to test geometric information without any distinctive features to act as landmarks. Participants were to learn how to navigate through hallways in a maze to escape as quickly as possible while having minimal errors. Results of the experiment indicated that men navigated faster and made fewer errors compared to women. An important measure that the study took into account was the number of errors on the first trial. The results indicated that men and women did not differ in the number of errors on trial 1, indicating that subsequent errors were a result of the task and not due to any initial differences. Participants also underwent a series of additional spatial and verbal tasks. Results indicated that men performed significantly better on these spatial tasks and that their performance on these tasks positively correlated with their performance in the virtual maze.
Overall the findings of this study yielded a male advantage in spatial navigation of the virtual maze and additional spatial tasks, including mental rotation, which has been seen in previous studies. This study focused on how males made fewer errors than females even though both groups had the same amount of initial errors, demonstrating that the overall performance was based on task learning (Moffat et al., 1998).

Another group of researchers aimed to investigate if similar results would occur if men and women navigated in a 3D model that had both geometric and landmark information (Sandstrom et al., 1998). In this study, participants completed a virtual version of the Morris water maze with different conditions. The conditions were as follows, stable landmark: landmarks available (no geometric information); geometric: only geometric features were available (no landmarks); and random landmark: geometric information was available along with landmarks that could be moved to random locations. Men and women performed at the same level during the stable landmark condition. However, men performed better than women in the geometric and random landmark conditions. Better performance was indicated by faster latency to complete trials, more trials in which the platform was found, and more direct pathways to the platform. The overall results of the study emphasize that females were negatively affected by the absence of stable and reliable landmarks. When this information was reliably available (stable landmark condition), women performed just as well as men. Thus, it seems as though men were more flexible in their approach to navigating in this task, whereas women were utilizing landmarks to complete the objective (Sandstrom et al., 1998). However, the researchers mention that their geometric information was not as salient in the maze as the landmarks were. If the geometric information was more salient, it is possible that a deficit in male performance could have occurred, had they more readily relied on that information to complete the task.
More recent research has focused on identifying why males and females perform so differently on tasks such as the Morris water maze that employs specific spatial navigation skills. Woolley and colleagues (2010) aimed to examine sex differences on a modified version of the task that allowed for careful consideration of the initial trajectory and later search phases. The results indicated that the most substantial sex difference was observed during the initial trajectory phase with men performing better than women. Results indicated that both sexes perform fairly similarly during the search phase. According to the researchers, the initial phase depends on efficient processing of distal features of the entire environment and not as much reliant on processing landmark features (Woolley et al., 2010). These results support prior research in which men have an advantage in processing geometric information.

The research reviewed demonstrates a clear sex difference in spatial navigation, partly due to differences in processing different kinds of available spatial information. The outcomes of these studies partially mimic the explanations of the hunter-gatherer theory. In both cases, males are more reliant on global, geometric, and distal features while females focus more on specific objects/landmarks to successfully navigate. Given that similar results were found between the spatial processing theory and hunter-gatherer theory, it is important to understand that both theories still disregard other factors that are related to sex differences such as the effects of hormones, hippocampal processing, and spatial experience.

1.5.3 Spatial Sex Differences in Children: Although spatial sex differences are apparent in adults, it is unknown when they become differentiated. If sex differences in spatial processing occur during early development, it would be evident in young children. Researchers have attempted to identify the period of time in which sex differences in spatial navigation occur. Many have examined children of various ages and have yielded unique and equivocal outcomes.
In one study, researchers investigated how children perform on a variety of spatial tasks (Choi & Silverman, 2003). Young children were between the ages of 9 and 13 years old. Participants were given a study page that contained an array of objects. For an object memory task, participants were tested with an array of objects that contained items from the study page and novel items. For a location memory task, participants were tested with an array of objects from the study page in which some had different locations. A spatial relations task was also administered that was a 2-D version of the mental rotation task. Finally, participants were given maps and had to indicate and memorize specific routes. Results indicated that girls outperformed boys on the object memory task, indicated by a greater number of correct items. There were no sex differences on the object location memory and spatial relations tasks. On the route learning task, 12 and 13 year old girls had a higher preference for using a landmark strategy than boys (Choi & Silverman, 2003). The findings of this experiment indicated that although girls had an advantage for object memory at a young age, most of the other tasks did not show a sex difference. Girls also only displayed a preference for landmarks at the highest age groups (12 and 13 year olds). The researchers then replicated the same experiment with adolescents between the ages of 14 and 17 years old. Results indicated that girls outperformed boys on the object memory and object location tasks whereas boys outperformed girls on the spatial relations task. Additionally, all age groups of girls showed a preference for landmarks on a route learning task. Overall, this study emphasized the distinctions in age specific sex differences of spatial processing. Object memory was found to have a sex difference at the young age of 9 years old, with a bias towards girls. However, the landmark preference of girls was not evident until around 12 or 13 years old and older. Furthermore, boys had the strongest advantage than girls on spatial relations such as mental rotation at the age of 15 years old and older. These results depict ages in
which adolescents typically hit puberty – around 12 years old for girls and around 14 years old for boys (Choi & Silverman, 2003).

The outcomes of the study by Choi and Silverman (2003) emphasize how spatial memory performance may be impacted by the initiation of puberty and the resulting changes in hormones. The strongest sex differences were observed during and post-puberty in which hormone levels may have the capability to impact and mediate spatial memory. However, other studies have not been able to demonstrate the same patterns of behavior across childhood and adolescence. This may be a result of the type of spatial memory that is being targeted. Researchers examined spatial memory performance of children aged 8 to 10 years old on a virtual Morris water maze, a task that is more reliant on navigation skills (Newhouse et al., 2007). Results indicated that boys performed better than girls indicated by shorter latencies to the platform and a greater percentage of distance in the quadrant that previously held the platform during a probe trial. The researchers postulate that the sex differences on this task may be due to differences in hippocampal functioning (Newhouse et al., 2007).

In addition to pubertal changes, childhood experiences may also contribute to early sex differences in spatial processing. Girls and boys are not always exposed to the same types of motivation, support, and inspiration (Shapiro & Williams, 2011). Girls are often presented with stereotypical and archaic examples of women that are often discouraging from science, technology, engineering, and math (STEM) fields. On the contrary, boys are often pushed towards STEM careers and are encouraged to explore these fields at young ages (Shapiro & Williams, 2011). It has been shown that adolescent and young adult students who are well versed in STEM fields typically have better spatial skills (Carr et al., 2017). Given these circumstances, it remains a possibility that lack of exposure to STEM fields may result in disadvantages on
spatial navigation tasks for females. Studies have shown that neural codes for spatial skills overlap with specific mathematical functions related to numbers (Carr et al., 2017). Additionally, there is a positive correlation between strong spatial skills and math achievements for children in elementary school (Carr et al., 2017).

Overall, the studies examining sex differences in spatial behavior in children yield conflicting and unclear results. In some cases, sex differences are heightened at the ages when puberty occurs. In other cases, sex differences were evident in pre-pubertal children on navigation tasks. Furthermore, findings were reliant on type of task and administration. Given prior research, the development of sex differences in children remains unclear.

1.6 Estradiol’s Influence on the Hippocampus

Many theories attempt to explain why and how sex differences occur. One of the most prevalent theories postulates that sex hormones contribute to differences in spatial memory performance (Daniel, 2013; Duarte-Guterman et al., 2015; Gould et al., 1990; Isgor & Sengelaub, 1998; Roof & Havens, 1992; Spencer et al., 2008; Woolley et al., 1990; Woolley & McEwen, 1992). Given that the hippocampus is prone to plasticity, undergoes neurogenesis, and contains many sex hormone receptors including ERα, ERβ, and GPER, all of which are estrogen receptors (Choleris et al., 2018; Duarte-Guterman et al., 2015), it is not surprising that estrogens have a substantial role in regulating hippocampal functions and in impacting cognitive processes such as learning and memory (Choleris et al., 2018; Frick 2015; Galea et al., 2016; Luine, 2014). The major type of estrogen hormone, estradiol, is most active during years in which reproduction in most probable in women (Sherman & Korenman, 1975). The primary estradiol hormone is 17β-estradiol, which has been shown to be effective in enhancing behavior and cognition based on its regulatory role in the hippocampus (Gillies & McArthur, 2010).
1.6.1 Effects on Hippocampal Structure. Early researchers were able to uncover how estradiol specifically induces structural changes to hippocampal dendritic spines and synapses (Gould et al., 1990; Woolley et al., 1990; Woolley & McEwen, 1992). In one experiment, researchers tested the effects of ovariectomized (OVX) female rats receiving either estradiol treatment or a vehicle (Gould et al., 1990). Results indicated that the OVX rats treated with estradiol had a 35% increase in apical spines in CA1 and a 28% increase in the number of synapses on those spines compared to OVX rats treated with vehicle (Gould et al., 1990; Woolley & McEwen, 1992). In another experiment, researchers examined the natural fluctuation of estradiol across an estrous cycle (Woolley et al., 1990). Rats in the proestrus phase of their natural estrous cycle, during which estradiol levels are high, had a 32% increase in the number of apical spines and a 23% increase in the number of synapses on those spines in CA1 compared to rats in the estrus phase, during which estradiol levels are low (Woolley et al., 1990; Woolley & McEwen, 1992).

In another experiment, these researchers aimed to understand how progesterone impacts the effects of estradiol due to variations in progesterone across the 5-day estrous cycle in addition to fluctuations of estradiol (Woolley & McEwen, 1993). During the proestrus phase there is a burst of progesterone released after peak estradiol levels are attained. Between the proestrus and estrus phase, progesterone levels rapidly decrease to baseline levels. The researchers aimed to identify the latencies of dendritic changes that occur in response to an ovariectomy and to estradiol and progesterone treatment. Results indicated that estradiol levels decrease dramatically following the OVX procedure. However, dendritic spine densities gradually declined over a period of 6 days. There was approximately a 25% decrease in density between days 1 to 6. With treatment of estradiol, the initial deficit induced by ovariectomy could
be reversed across a 6-day period (32%) and a 12-day period (42%) compared to OVX rats treated with vehicle. Increases in dendritic spine density induced by estradiol began within 24 hours of administration and reached peak levels within 48 hours. Finally, the impact of progesterone on estradiol’s actions was tested and results indicated that progesterone initially aids estradiol in increasing spine density and then subsequently is involved in dramatically lowering dendritic spine densities when the estrus phase is reached (Woolley & McEwen, 1993). Across this set of experiments, the findings make a strong case for estradiol’s role in mediating structural alterations to synaptic and spine density. Additionally, progesterone was shown to enhance the impact of estradiol on hippocampal alterations. Because there were changes in hippocampal synapses and spine density, there is an implication that estradiol has an important regulatory role in hippocampal functions, especially in learning and memory for females.

Numerous additional experiments have examined how estradiol influences hippocampal morphological changes. One study examined how administration of varying doses of estradiol affected hippocampal dendritic synapses in OVX rats (MacLusky et al., 2005). OVX rats treated with a low dose (15 µg/kg) of estradiol had no changes in dendritic spine synapses and were comparable to OVX rats that received a vehicle. In contrast, OVX rats that received a medium (45 µg/kg) or high (60 µg/kg) dose of estradiol had a 44% and 65% increase in CA1 dendritic spine synapses, respectively (MacLusky et al., 2005). Similarly, another study found a 29% increase in spine density of dendritic spines in CA1, 30 minutes post-injection of estradiol in OVX rats (Phan et al., 2012). In both experiments, the changes in dendritic structure remained for approximately 4 hours post-administration of estradiol.

1.6.2 Effects on Hippocampal-Dependent Behavior. Given that estradiol impacts hippocampal morphology, studies have examined how estradiol’s effects can directly impact
hippocampal-dependent behavior in animals. One study observed differences in structural size and thickness of hippocampal cell layers that contributed to sex differences in water maze navigation behavior (Roof & Havens, 1992). The researchers examined performance of male and female rats in the Morris water maze. The results indicated that male rats performed better than female rats on the task and that the granule cell layer of the DG was larger in males than females. Furthermore, there was a strong positive correlation ($r = .86$) between performance on the maze and the width of the granule cell layer of the DG in male rats (Roof & Havens, 1992).

In another study, researchers assessed sex differences in hippocampal neurogenesis in response to training on a spatial task (Chow et al., 2013). Male and female rats were injected with a DNA synthesis marker. Six to ten days post-injection, rats were trained on a spatial (hidden platform) or cued (visible platform) version of the Morris water maze. Twenty days post-injection, rats were tested in a probe trial and perfused. Results indicated that male rats performed better on the spatial task than females, but no difference occurred in the cued condition. Within males, spatial training significantly increased neurogenesis compared to cued training. However, both male and female rats showed greater activation in new cells in response to spatial training. Additionally, performance during spatial training was positively correlated ($r = .82$) with cell activation in females but not in males. In examining menstrual cycle phase in female rats, results indicated the proestrus females that underwent the spatial training had better performance (greater percentage of time in target quadrant) compared to female rats in the estrus cycle during the probe trial (Chow et al., 2013). Overall, the findings of this study show that spatial training impacts hippocampal neurogenesis and that there are sex differences in cell activation in response to a spatial memory task. These results can be extended to work done with OVX rats as well. Estradiol administration in OVX female rats improved hippocampal-
dependent spatial memory (Luine et al., 1998; Packard, 1998; Sandstrom & Williams, 2004), working memory (Bimonte & Denenberg, 1999; Luine et al., 1998), object recognition and object place recognition (Jacome et al., 2010; Luine, 2014), compared to non-treated OVX rats. In these studies, higher levels of estradiol led to improvements or better performance on a variety of behaviors including spatial memory performance. This demonstrates that elevations in estradiol are not only beneficial for spatial memory during low phases of natural fluctuations across an estrous cycle (Chow et al., 2013) but also in OVX animals (Bimonte & Denenberg, 1999; Jacome et al., 2010; Luine, 2014; Luine et al., 1998; Packard, 1998; Sandstrom & Williams, 2004). Collectively, prior work has shown that higher levels of estradiol were associated with better spatial memory than lower levels of estradiol in females.

Studies done with humans have revealed a male advantage on a variety of hippocampal dependent tasks, including spatial navigation tasks such as virtual mazes (Astur et al., 1998; Moffat et al., 1998; Sandstrom et al., 1998; Woolley et al., 2010) and map route learning or wayfinding tasks (Galea & Kimura, 1993; Postma et al., 2004). One study examined the effect of estradiol treatment on a variety of memory tasks (Bartholomeusz et al., 2008). Women between the ages of 18 and 38 with typical menstrual cycles were administered 100 µg of estradiol a day via transdermal skin patches for 31 days. Participants monitored their menstrual cycle and began use at the start of their menstrual cycle (early follicular phase). It was expected that this dose would elevate circulating plasma estradiol levels to around 75 pg/ml (Bartholomeusz et al., 2008). This dose is relatively low considering the minimum level of estradiol is approximately 109 pg/ml and occurs during the early follicular phase whereas the maximum level is around 578 pg/ml that occurs during ovulation (Leonard, 2004). Participants were tested on a spatial working memory version of the N-back task in which they had to indicate whether an object rapidly
shown was in the same or different location as the proceeding object (Bartholomeusz et al., 2008). Results on this task indicated that performance was enhanced in the estradiol-treated group compared to controls. The estradiol-treated group also performed better on recollection following a long-delay on a verbal learning and memory task. Other tasks such as verbal fluency, attention on the Stroop task, and information processing were unaffected by estradiol administration (Bartholomeusz et al., 2008). Results of this study emphasize the importance of elevated estradiol levels on spatial working memory and some aspects of verbal memory.

For comparisons across naturally fluctuating levels of estradiol, many studies (Bayer et al., 2014; Bartholomeusz et al., 2008; Protopopescu et al., 2008) have found a relationship between hippocampal changes and behavioral performance across the menstrual cycle in women, while some have not (Phillips & Sherwin, 1992; Resnick et al., 1998). One study examined the variation in hippocampal gray matter across the menstrual cycle of women aged 22 to 35 (Protopopescu et al., 2008). Performance on a verbal declarative memory task was also assessed in which participants were shown a list of previously seen words with distractor words and had to identify which they had seen before (test of recognition). Women in the late-follicular phase, during which estradiol levels are at peak elevation, performed better on this task than women in the late-luteal phase, during which estradiol levels are relatively low. Results also indicated that gray matter was relatively increased in the right anterior hippocampus in the late-follicular phase, and relatively decreased in the dorsal basal ganglia. Furthermore, this change in the hippocampus was associated with behavioral performance. Results indicated that there was a positive correlation between late-luteal to late-follicular improvement in verbal memory performance and between late-luteal to late-follicular increases in gray matter of the right anterior hippocampus (Protopopescu et al., 2008).
Even more studies have examined how behavioral patterns fluctuate across the menstrual cycle. In one study, researchers tested men and women between the ages of 17 and 37 on a spatial working memory task (Hampson & Morley, 2013). Participants were to find 10 matching pairs of colored dots hidden behind doors on a board. Three trials were administered consecutively followed by a delayed trial approximately 30 to 40 minutes later. Results showed that women with high estradiol levels (based on salivary assay) produced the least amount of errors across all trials compared to women with low levels of estradiol. There was no sex difference between number of errors men and women with high levels of estradiol produced during the delayed trial (Hampson & Morley, 2013). Other researchers have found that women in the mid-luteal phase, during which estradiol levels are medium to high, performed better on the Stroop task compared to women during menses/early follicular phase (Hatta & Nagaya, 2009). Solis-Ortiz and Corsi-Cabrera (2008) examined how performance on a visuospatial and a verbal memory task was affected by cycle phase. The visuospatial task was a localization task in which participants were shown a shape on a blank screen for a brief period and had to mark the location of the object. This was repeated for 24 trials with different object positions. The task score was based on linear error in millimeters. The verbal memory task was verbal fluency in which participants had to spontaneously generate words that begin with specific letters. Results indicated that women in the ovulatory phase, during which estradiol levels are relatively high, had enhanced performance on the visuospatial task (less error in distance to the objects) compared to women in the late-luteal and menstrual phases of their cycles. Women in the ovulatory group also produced fewer words on the verbal fluency task compared to the women in the late-luteal phase (Solis-Ortiz & Corsi-Cabrera, 2008).

Additionally, another study found that an ovulatory group performed significantly better
than women in other phases on the Rey Auditory Verbal Learning Test (RAVLT), a standard measure of verbal memory recollection, but no group differences occurred on a radial arm maze (Hussain et al., 2016). The researchers also discussed how progesterone plays an important role in interacting with estradiol’s effects on learning and memory. In rats, both hormones peak in the same phase (proestrus), although there may be a delay of a few hours before the spike in progesterone. In humans, estradiol and progesterone peak independently (Hussain et al., 2016). Thus, given prior research that has shown that progesterone tends to enhance the effects of estradiol, the timing of peak levels can heavily impact the relationship these hormones have in regulating the hippocampus. It can be assumed that if progesterone enhances the effects of estradiol, women would have enhanced spatial memory performance during the luteal phase of the menstrual cycle during which both hormones are relatively high. However, it is possible that estradiol may have different effects on human hippocampi due to the independent peaking of progesterone that is not seen in rodent models.

**1.6.3 Effects of Oral Contraceptives.** In addition to natural fluctuations of estradiol, it is also important to consider whether synthetic estradiol in an oral contraceptive (OC) impacts cognition. According to the Centers for Disease Control, 61% of women aged 15-44 used OCs during the period from 2011 to 2013 (Daniels et al., 2014), and these percentages remain similarly high in subsequent years. Furthermore, 88% of US women have used a reversible hormonal contraceptive, and 85% of women typically use an OC for around 5 years at some point in their life (Chadwick et al., 2012). OCs contain different forms of synthetic estradiol (e.g., ethinylestradiol) and progesterone (progestin) that bind to the same estrogen and progesterone receptors as endogenous hormones (Gogos et al., 2014; Griksiene & Ruksenas, 2011; Rivera et al., 1999). These synthetic hormones also have different pharmacokinetic
properties. For example, ethinylestradiol has greater bioavailability and is slower to be metabolized than 17ß-estradiol (Kuhl, 2005). Given that these synthetic forms of hormones are taken exogenously into the system, the effects on hippocampal cognition may be different than endogenous estradiol that is synthesized internally (Beltz et al., 2015; Gogos et al., 2014; Griksiene & Ruksenas, 2011; Mordecai et al., 2008).

The most common types of OCs inhibit conception by decreasing the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH; Rivera et al., 1999). In a natural menstrual cycle, LH and FSH are secreted concurrently to stimulate a rise in estradiol that induces ovulation. Thus, OCs prevent ovulation by inhibiting gonadotropin release, decreasing estradiol levels, and altering the physical lining of the uterus to prevent implantation (Rivera et al., 1999). An increase in exogenous estradiol and progesterone inhibits the release of endogenous estradiol and progesterone via the hypothalamic-pituitary-gonadal axis (Beltz et al., 2015; Gogos et al., 2014; Griksiene & Ruksenas, 2011). One study quantified this suppression of endogenous hormones by comparing hormonal levels in women taking OCs and in naturally cycling women (Mordecai et al., 2008). As reported from serum analyses of women tested at the same phase in their cycles, the average level of estradiol in OC users was 16.96 pg/mL compared to 62.93 pg/mL in naturally cycling women. The average progesterone level in OC users was 0.55 ng/mL compared to an average level of 3.93 ng/mL in naturally cycling women (Mordecai et al., 2008). The findings from this study emphasize how OCs suppress natural levels of endogenous hormones.

1.6.4 Hormone Replacement Therapy. As research has shown, higher circulating levels of estradiol have been shown to be beneficial across a variety of tasks in both non-humans and humans. Women typically hit the stage of menopause around the ages of 51 to 53, which marks
the cessation of the menstrual cycle and results in large reductions in gonadal hormones including estradiol (Birkhauser et al., 2000; Duff & Hampson, 2000; Gold et al., 2013; Sherwin, 2007; Simpkins et al., 1997). Given this, women spend around one third of their life deprived of circulating estradiol (Birkhauser et al., 2000; McCarrey & Resnick, 2015; Simpkins et al., 1997). Many believe that hormone replacement therapy (HRT) can be beneficial in combatting the negative ramifications that occur during menopause (Birkhauser et al., 2000; Brinton, 2004; Costa et al., 1999; Duff & Hampson, 2000; Gibbs, 2010; Maki et al., 2001). HRT can be given by oral or transdermal methods that include synthetic variations of estradiol alone or in combination with synthetic progesterone (Maki et al., 2001; Sherwin, 2007).

In one study, researchers tested the effects of estradiol administration in aged mice as a model for post-menopausal women (Frick et al., 2002). Aged mice undergo a similar phase to menopause in women in which there are rapid reductions in levels of estrogen and progesterone (Frick et al., 2000). The reductions can start as early as 17 months and the estrous cycle typically has ceased by 25 months of age (Frick et al., 2000). Thus, Frick and colleagues (2002) chose to examine mice between 27 to 28 months old. Aged mice were given injections of either a 1 µg or 5 µg dose of estradiol or a vehicle daily. Treatment began 5 days prior to behavioral testing and continued throughout. Mice were tested on a spatial and non-spatial version of the Morris water maze. Results indicated that mice treated with the 5 µg dose of estradiol improved spatial learning and memory compared to controls across trials for both swim time and swim distance to the platform. There was no behavioral difference on the spatial task between the mice treated with the 1 µg dose of estradiol and control mice. Furthermore, no doses of estradiol affected performance on the non-spatial task (Frick et al., 2002).

In other studies, ovariectomization has often been used as a model of post-menopausal
women given the drastic cessation of hormone levels that occurs in both cases. As previously discussed, estradiol administration in OVX rats can lead to changes in hippocampal structure (Gould et al., 1990; Woolley et al., 1990; Woolley & McEwen, 1992; 1993). One study found that when OVX female rats were given a moderate dose of estradiol, they made fewer errors on a water-escape version of the radial arm maze that examined spatial working memory, compared to OVX female rats that were given a low dose of estradiol, OVX female rats that received no estradiol, and gonadally intact female rats (Bimonte & Denenberg, 1999). Another study tested the effects of acute and extended use of estradiol treatment in OVX female rats (Sandstrom & Williams, 2004). Rats were behaviorally assessed on a delayed matching-to-place version of the water maze in which the platform was randomly located in the pool for pairs of trials but remained at the same location between training and test trials within a pair. When estradiol was administered for two consecutive days (acute treatment), these rats showed improvements in memory retention up to 4 days post-treatment compared to OVX rats that received a vehicle. When rats were treated with estradiol for 10 consecutive days (extended treatment), memory improvement persistently for the entire 10 days. Furthermore, the rats with extended treatment performed better than controls during the first 4 days and then better than controls and the acute treated rats from days 5 to 10 (Sandstrom & Williams, 2004). This study shows that long-term use of estradiol replacement can sustain memory enhancement.

Research has also examined how progesterone impacts the effects of estradiol treatment (Gibbs, 2000). In this study, OVX female rats received hormone treatments at various time points following surgery for a total of 5 groups. One group received estradiol immediately following the surgery. Another group received estradiol 3 months post ovariectomy. Another group received both estradiol and progesterone 3 months post ovariectomy. One group received
both estradiol and progesterone 10 months post ovariectomy. Finally, the control group was OVX female rats that received no hormonal treatment. Female rats were assessed on a delayed matching-to-position spatial memory task (paired with a T-maze) 8 to 12 months after the ovariectomy. Control rats that received no hormonal treatment required more days to reach criterion than all other groups. Rats that received both estradiol and progesterone 3 months post-surgery required the least number of days to reach criterion. All groups treated with hormones improved at a faster rate on the task than the control group. Overall performance was the best for the group that received both estradiol and progesterone 3 months post-surgery. The next best groups were the rats treated with estradiol only either immediately or 3 months after ovariectomy. The group that received estradiol and progesterone at 10 months post-surgery performed the next best followed by the control rats that performed the worst (Gibbs, 2000). The results from this study indicate that not only are hormone treatments effective in enhancing behavior but that timing is key. OVX rats treated earlier on (either immediately or 3 months post-surgery) performed better than those treated at 10 months after surgery. This may be paralleled in post-menopausal women who undergo hormone replacement therapy. It is likely that earlier treatment will lead to better outcomes than for those who do not start HRT until much later. Additionally, this study emphasizes the important role that progesterone had in enhancing the beneficial effects of estradiol. When rats received both hormones, they performed better than rats that received only estradiol.

In human studies, many have examined how HRT impacts cognition in post-menopausal women. In one study, researchers examined how HRT impacted spatial working memory and verbal working memory (Duff & Hampson, 2000). Post-menopausal women were already receiving treatment either with estrogens only, estrogens plus progestin, or no hormonal
treatment. For the spatial working memory task, participants were to find 10 matching pairs of colored dots hidden behind doors on a board for two consecutive trials. For the verbal memory task (digit ordering), participants were asked to say the numbers 1 to 10 in a random order aloud without repeating or missing any digits for a total of 10 trials. Results indicated that women who received either hormonal treatment had fewer working memory errors on the verbal task compared to the control group without hormonal treatment. Similarly, results on the spatial working memory task showed that women with either hormonal treatment had fewer working memory errors and faster completion times compared to the control women (Duff & Hampson, 2000). Many others have shown that hormone replacement therapy was effective in improving learning and memory in women compared to no treatment. HRT was shown to enhance verbal memory on the California Verbal Learning Test in post-menopausal women taking HRT either with estrogen alone or in conjunction with progesterone (Maki et al., 2001; Resnick & Maki, 2001). Estrogen placement also improved verbal recall in post-menopausal women compared to control women not receiving any hormonal treatment (Robinson et al., 1994).

1.6.5 Estradiol and Alzheimer’s Disease. Many researchers have examined the association between menopause and dementia in aging women. Women are two times more likely to develop Alzheimer’s disease (AD) than men (Herbert et al., 2013). Patients with AD exhibit a variety of cognitive deficits including progressive loss of memory, confusion, disorientation, and much more (Lyketsos et al., 2011; Merriam et al., 1988; Reisberg et al., 1987). Research has shown that women who undergo menopause earlier than the average age have a higher risk for developing AD (Depypere et al., 2016). Furthermore, Fox and colleagues (2013) revealed that the longer the exposure to estrogen hormones a woman has the more protective effects it has against the risk for developing AD. Specifically, they calculated that for
every additional month with circulating estradiol, women have an approximate 0.5% decrease in the chance of having AD (Fox et al., 2013). Studies have investigated the role that estradiol may play in mediating cholinergic neurons that may also be involved in neurodegeneration. It was theorized that gonadal hormones, particularly estrogen hormones, may increase production of choline acetyltransferase (ChAT), which synthesizes acetylcholine (Depypere et al., 2016; Gibbs, 2010; Luine, 1985; Mielke et al., 2014). Reductions in ChAT and acetylcholine have been linked to the development of AD. Furthermore, the hippocampus receives direct cholinergic inputs (Gibbs, 2010). Overall, it appears that the drastic reductions in ovarian hormones that occur during menopause are related to cognitive processing and may impact the likelihood of developing dementia.

One study examined the effects of estrogen replacement on behavioral performance and cholinergic neuronal loss in OVX rats (Simpkins et al., 1997). Gonadally intact rats, OVX rats, and OVX rats that received estradiol treatment were tested on the Morris water maze and an active-avoidance paradigm. On the Morris water maze, there were no differences between the three groups during acquisition trials. However, the intact rats and OVX that received estradiol treatment showed normal learning during the probe trial 24 hours later. The OVX rats without estradiol treatment had impaired performance during the probe trial. A similar pattern was seen for avoidance behavior in that the OVX group performed worse compared to both other groups (Simpkins et al., 1997). These results indicate once again that estradiol treatment was beneficial in preventing potential impairments from estradiol reductions in females. These researchers also investigated how estradiol affected cholinergic neurons in OVX rats. They observed that 5 weeks after an ovariectomy, OVX rats had a decrease in high affinity choline uptake by 24% in the hippocampus and 34% in the frontal cortex. ChAT activity was also reduced in the hippocampus.
in OVX rats. Estradiol replacement was able to negate both of these deficits. The researchers suggest that cholinergic neurons not only respond to changes in estrogen hormones but that continuous exposure to estradiol may be necessary for normal functioning of the cholinergic system. Based these findings, the researchers also suggest that estradiol therapy may be useful in treating or preventing Alzheimer’s disease given its interaction with cholinergic neurons (Simpkins et al., 1997).

Another study examined how estrogen replacement impacted the diagnosis of AD and cognitive impairment based on a dementia rating scale in post-menopausal women across a one-year period (Costa et al., 1999). All participants were initially part of a treatment center based on general memory impairments. Results indicated that women who were on estrogen replacement had significantly lower rates of AD diagnosis than women not on hormonal treatment at baseline. Furthermore, women on estrogen treatment had higher cognitive functioning based on the rating scale at both baseline and at the one year follow up. Women not on estrogen treatment had significant deficits in cognition from baseline to follow up, whereas estrogen treated women had no differences between the two points in time (Costa et al., 1999).

Other research has focused on the apolipoprotein E-ε4 gene (APOE-4) that is a genetic risk factor for AD (Corder et al., 1993; Tsai et al., 1994). Women with the APOE-4 gene have a higher risk for developing AD than men and some believe that it has to do with an interaction with estrogen hormones (Farrer et al., 1997; Neu et al., 2017). Yaffe and colleagues (2000) examined the cognitive decline in post-menopausal women with estrogen replacement, the APOE-4 gene, and the risk of developing AD. Participants completed the Modified Mini-Mental State Examination, which is more sensitive to milder cognitive impairments, every year for 5 to 7 years. Participants were either using estrogen replacement during this period, had previously
used it, or had never used it. Results indicated that at a 6 year follow up, non-estrogen users had a greater decline in cognition compared to baseline, than estrogen users. Additionally, in women without the APOE-4 gene, estrogen treatment reduced the risk of cognitive impairment compared to non-users by almost half. In contrast, there was no reduction of risk in women with the APOE-4 gene (Yaffe et al., 2000). The findings of this study suggest that while estrogen treatment may lessen cognitive decline over time, it may only be beneficial for women who do not have the APOE-4 gene.

Across myriad of research, it is evident that sex differences occur in spatial memory domains. Furthermore, prior work has shown that estradiol impacts learning and memory, and for the most part tends to enhance behavior. However, what remains unclear is how other factors influence the underlying mechanisms of spatial learning and memory.

1.7 Impacts of Anxiety and Stress on Spatial Memory

Women have an increased risk for developing stress, anxiety, and depressive disorders compared to men (Altemus et al., 2014; Kessler et al., 2005; Maeng & Milad, 2016; Newhouse & Albert, 2015). Specifically, women have a lifetime rate of depression and most anxiety disorders for twice as long as men (Altemus et al., 2014). Many theories have attempted to explain this discrepancy. Some believe that since women experience more sensitivity to rejection, criticism, and separation that they are more likely to develop these disorders (Cyranowski et al., 2000; Martel, 2013; Zahn-Waxler et al., 2008). However, environmental influences such as cultural gender stereotypes may also play role and these environmental factors may also alter gene expression (Carter et al., 2011). Additionally, fluctuations in gonadal sex steroids and stress responses expose women to disturbances on a regular basis (Altemus et al., 2014). Women also experience symptoms with more severity and to a higher degree that persist
for longer than typically in men (Altemus et al., 2014; Maeng & Milad, 2016). For example, women with generalized anxiety disorder experience fatigue, muscle tension, cardio-respiratory, and gastrointestinal symptoms more often and with more severity compared to men (Altemus et al., 2014). Furthermore, women are more likely to have a comorbid mood disorder such as major depressive disorder (Altemus et al., 2014).

Given that men and women may experience stress and anxiety differently, and that the hippocampus can result in changes due to learning and memory, it is important to understand how stress and anxiety may impact hippocampal-dependent memory. While there is a distinction between stress and anxiety, the two are related and often co-exist. Stress typically refers to a physiological reaction within the body to life events (Haynes, 1974). Anxiety typically refers to a state in which an individual has repetitive worry and negative thoughts about situations (Lader, 2015; Wolpe, 1958).

1.7.1 Anxiety, Memory, and the Hippocampus. Anxiety can be further defined as a response to potential danger and involves preventing the organism from being exposed to potentially dangerous situations (Bannerman et al., 2014). Anxiety often occurs when there is conflict or uncertainty about multiple choices than can be made (Bannerman et al., 2014; Gray & McNaughton, 2000). Furthermore, some researchers suggest that anxiety may impact spatial memory by hippocampal processes (Gray & McNaughton, 2000). Research has shown that individuals who suffer from anxiety have more difficulty in forming new memories, have impaired learning, and have deficits in memory consolidation (Maeng & Milad, 2016).

Researchers tested the effect of anxiety on behavioral performance of working memory tasks (Ikeda et al., 1996). Participants were grouped into high anxious and low anxious based on test anxiety, defined as worry and concern. Participants were then behaviorally assessed on a
verbal task in which they were shown written words and were to memorize the combination of words in each pattern. Following that, recognition sessions began in which patterns were either previously seen or never seen and participants were to identify the patterns they remembered from the learning trials. Participants also completed a spatial task in which drawings were presented. Following the learning trials, recognition sessions began in which some drawings were old, and some were new. Participants were to identify the old ones. The highly anxious group expressed more worry and self-concern than the low anxiety group in subjective measures. The high anxiety group also had longer reaction times on the verbal working memory task compared to the low anxiety group. However, there was no group difference on the spatial working memory task (Ikeda et al., 1996). Results from this study show that task anxiety led to slower reaction times on a verbal memory task. However, it is unclear whether delayed reaction times on a verbal task can be considered impairment in memory.

One study examined sex differences in anxiety and spatial memory (Lawton, 1994). Participants were tested on a wayfinding task in which either a route learning or orientation strategy could be used while driving. A route learning strategy focuses on specific instructions from place to place, whereas an orientation strategy monitors one’s own position relative to reference points in an environment. Results indicated that men were more likely to use an orientation wayfinding strategy and women were more likely to use a route wayfinding strategy. Furthermore, women experienced higher levels of spatial anxiety compared to men as assessed by the Spatial Anxiety Scale. Additionally, orientation strategy use was negatively correlated with spatial anxiety ($r = -.15$) across both sexes. In examining that correlational relationship within each sex, orientation strategy use was not correlated with spatial anxiety in women,
however there was a negative correlation in men ($r = -.20$). Given that men experienced lower levels of spatial anxiety, it is possible that this helped their spatial performance (Lawton, 1994).

In a similar study, Schmitz (1999) observed sex differences in wayfinding behavior that was related to spatial anxiety and self-confidence. Participants navigated in unfamiliar environments and then explained wayfinding directions based on either a map or description of the environment. Spatial anxiety was also assessed on a subjective rating scale. Results indicated that men had better memory for directions and descriptions of various routes than women. Men also preferred route directions and map representation strategies while women preferred landmark strategies. Men displayed higher self-estimation of task competence which was positively correlated with faster task performance ($r = .74$). Women had higher levels of spatial anxiety which was positively correlated with landmark strategy use ($r = .66$). As seen in previous work, men outperformed women on a spatial navigation task and women displayed a higher level of spatial anxiety which may be involved in hindering spatial performance (Schmitz, 1999).

Research has also examined how estrogen hormones impacts anxiety and behavior. Walf and colleagues (2009) examined how estradiol replacement therapy impacted spatial memory and anxiety in OVX female rats. Rats were either ovariectomized at 14 or 19 months. Rats either received estradiol implants at the time of surgery or after a 5-month delay. The control group consisted of rats that had surgery at 14 months of age and received no estradiol treatment. Rats were behaviorally examined at 20 months of age. On an object placement task, OVX rats that received estradiol treatment at any point performed better than OVX without treatment. To measure anxiety, rats were assessed in an open field and forced swim task. On both tasks, OVX rats that received immediate estradiol had reductions in anxiety-like behavior compared to OVX rats that received delayed treatment or no treatment at all (Walf et al., 2009). The findings from
this study indicated that estradiol treatment was able to improve both spatial memory and reduce anxiety in OVX female rats. However, it was evident that for some tasks, the timing of estradiol treatment was instrumental in impacting behavior. Receiving early estradiol treatment was beneficial for anxiety measures but did not impact behavior on a spatial object placement task since both treated groups performed similarly, regardless of the timing of treatment.

1.7.2 **Stress, Memory, and the Hippocampus.** As previously mentioned, stress refers to a physiological reaction within an organism in response to an event (Haynes, 1974). Stress can also describe experiences that are emotionally and physiologically challenging (McEwen, 2007). A stressful event will activate the sympathetic nervous system (SNS) and the hypothalamus-pituitary-adrenal (HPA) axis (Dickerson & Kemeny, 2004; Hidalgo et al., 2019; Kudielkaa & Kirschbaum, 2005; McEwen, 2007; Merz & Wolf, 2017; Stephens et al., 2016). The HPA axis releases corticotropin-releasing hormone from the hypothalamus to stimulate the release of adrenocortico-tropic hormone (ACTH) from the pituitary gland (Dickerson & Kemeny, 2004; Kudielkaa & Kirschbaum, 2005; Merz & Wolf, 2017). ACTH then synthesizes and secretes glucocorticoids from the adrenal cortex (Dickerson & Kemeny, 2004; Kudielkaa & Kirschbaum, 2005; McEwen, 2007; Merz & Wolf, 2017; Sapolsky et al., 2000). Glucocorticoids can enter the brain and impact brain regions involved in learning and memory such as the hippocampus, prefrontal cortex, and amygdala (Hidalgo et al., 2019; Merz & Wolf, 2017; Roozendaal et al., 2009). A stress response not only results in large secretions of cortisol, the main type of glucocorticoid in humans, but also releases catecholamines (Hidalgo et al., 2019; Merz & Wolf, 2017). The main type of catecholamine that is released is adrenaline, which is unable to enter the brain but leads to a series of steps that ultimately results in modifications in the hippocampus and prefrontal cortex (Hidalgo et al., 2019; Schwabe et al., 2012). The activation of the SNS and
HPA axis releases these hormones that are vital to health and survival (McEwen, 2007). Furthermore, in understanding an organism’s response to stress-invoking events, researchers often examine variations in cortisol level and activation of the SNS such as changes in heart rate. The assessments of these physiological reactions to stress are discussed below.

A plethora of non-human studies have examined a model of stress in rodents to explore the impacts on learning and memory. Numerous studies led by Victoria Luine and her colleagues have examined sex differences on spatial memory tasks following chronic exposure to stress in rodent models (Beck & Luine, 2002; Bowman et al., 2001; Luine et al., 1994; 1996). In a set of studies, the researchers found that male and female rats responded differently to chronic restraint stress and that behavioral performance on a radial arm maze was also differentially impacted (Bowman et al., 2001; Luine et al., 1994; 1996). Male rats that were exposed to 21 days of restraint stress had impaired performance compared to control male rats, indicated by a greater number of errors, on the radial arm maze (Luine et al., 1994; 1996). In contrast, female rats that were exposed to the same stressor also for 21 days had enhanced performance, with fewer errors and fewer visits to arms, compared to control female rats in the radial arm maze (Bowman et al., 2001). In a comparison review of these studies, it was observed that non-stressed males had fewer errors compared to stressed females (Bowman, 2005). However, stressed males and females had approximately the same number of errors. This is important to note because it seems evident that the impact of stress is dependent on the initial learning and memory (Bowman, 2005). The overall findings emphasize the detrimental effects of prolonged stress in male rats but introduce a possible advantage of chronic stress for female rats. The researchers suggest that a possible explanation for the sex difference they observed may be because female rats displayed adaptation or habituation to prolonged stress.
In addition to performance on the radial arm maze, the Luine group also examined how restraint stress for 21 days and housing conditions (paired or single housing) impacted performance on an object location task (Beck & Luine, 2002). Results showed that object location memory was impaired in stressed males that were singly housed, compared to stressed males that were paired housed, both groups of stressed females, and control males. Whereas stressed females were unaffected by housing condition and performed as well as control males and better than control females (Beck & Luine, 2002).

Other studies have shown that male rats exposed to chronic restraint stress (21 days) also have impairments on a Y-maze (Conrad et al., 2003) and Morris water maze (Kitraki et al., 2004) compared to control male rats. Furthermore, the stressed males that had behavioral impairments on the Morris water maze also had decreased glucocorticoid receptor binding for glucocorticoids in CA1 and DG, indicating a reduced response to stress hormones (Kitraki et al., 2004). In contrast, female rats have enhanced performance on the Morris water maze when exposed to the same stressor and had increases in glucocorticoid receptor binding in CA1, compared to control females (Kitraki et al., 2004). However, in using a different type of stressor, some researchers have found conflicting results in non-spatial task (Shors, 2001; Shors et al., 1998). In one study, Shors (2001) examined how a salient and repetitive stressor (tail shock) affected conditioned place preference in male rats. Results indicated that exposure to a 1-second, 1mA, 60 Hz tail shock (once a minute for 30 minutes) enhanced conditioning in that the rats were more likely to prefer the environment not paired with the shock. Furthermore, in testing different timings of the shock, the researchers found that exposure to the stressor only strengthened the formation of new associations. Retention of conditioning was not affected (Shors, 2001). Shors and colleagues (1998) conducted the same experiment with female rats and examined how
performance differed in the varying estrous cycle phases. These rats were exposed to the same tail shocks as the male rats in Shors (2001) study. Results indicated that control females in the proestrus phase had enhanced conditioning compared to control female rats in all other phases. However, exposure to the stressor impaired acquisition of conditioning in females during proestrus compared to females in the estrus phase (Shors et al., 1998). Thus, in response to fear conditioning, male and female rats had a different pattern of behavioral outcomes.

Exposure to stress can also lead to structural alterations of the hippocampus. In one study, researchers had male rats exposed to chronic random stressors (injection of hypotonic saline, overcrowding, restraint, and placement on a vibrating platform) for one month (Sousa et al., 2000). Results indicated atrophy in dendrites of CA3 and atrophy in granule and pyramidal cells in CA1 (Sousa et al., 2000). Chronic restraint stress (21 days) also led to reductions in cell proliferation in the DG, whereas acute restraint stress did not have any impact on cell proliferation in this region (Pham et al., 2003). However, one study found sex differences in how hippocampal spine density was affected by acute stress (Shors et al., 2001). Prior to stress, the researchers found that female rats in proestrus have a greater spine density in CA1 than male rats. However, following exposure to intermittent tail shocks, spine density increased in male hippocampi but decreased in female hippocampi (Shors et al., 2001).

Overall, the results of non-human studies done with male rats show a strong impairment for spatial memory in response to prolonged and chronic stress exposure that do not threaten survival. Conversely, female rats show either enhancement or no impact of chronic stress that do not threaten survival. However, when rats were exposed to a fearful stress (tail shock), males had enhanced conditioning. For the same stressor, females in the proestrus phase had impaired conditioning, compared to females in the estrus phase. It is possible that these findings are also
related to how stress hormones interact with hippocampal processing and may impact spatial memory (Bowman, 2005; Luine et al., 2017).

Studies done with humans have also revealed how stress hormones impact memory. In one study, participants were either given a placebo or 10 mg of cortisol, which the researchers considered to be a low dose, and were evaluated on a variety of tasks (Kirschbaum et al., 1996). For a declarative memory task, participants were presented with a list of words and asked to learn the list. Following a distraction, participants were to recall words that began with specific letters. For a spatial thinking task, participants read a description of a guided path with distinct directions and landmarks along the way. They were to memorize the guided path and had to answer questions related to the path, including events in which some landmarks had moved or were rotated. Results indicated that the cortisol group made significantly more errors on the declarative memory and spatial thinking tasks compared to control participants who received a placebo. Performance on the procedural memory task, in which participants were presented with a list of words and had to recall them in response to priming, was unaffected (Kirschbaum et al., 1996). The findings of this study suggest that by inducing elevations in cortisol levels, behavioral performance on some types of tasks can be affected.

Other studies examined sex differences in response to induced acute stress in humans. One study found that adolescent boys had greater cortisol elevations in response to the Trier Social Stress Task (TSST), in which a speech had to be given in front of authority figures, compared to adolescent girls (Mazurka et al., 2017). Similarly, other researchers also found that adult men had a greater elevation in cortisol in response to a socially evaluated cold pressor task, in which participants had to submerge their hand in ice water for a certain amount of time while being observed, compared to adult women (Guenzel, et al., 2014). In another study, researchers
examined the impact of induced stress on fear conditioning (Zorawski et al., 2006). They found that cortisol levels post-acquisition of fear was positively correlated ($r = 0.57$) with the conditioning response during acquisition in men but not in women. Furthermore, post-acquisition cortisol was also positively correlated with fear consolidation but only in men that yielded high cortisol levels and not for men with a low cortisol response. The researchers postulated that elevations in cortisol may only impact memory consolidation in those who have a high stress response (large increases in cortisol) to fear learning rather than in individuals that have a low stress response (Zorawski et al., 2006).

Andreano and Cahill (2006) tested how recollection performance in men and women was impacted by the cold-pressor task. Participants had to read a story which was immediately followed by the stressor. Participants returned a week later and recalled details from the story. Results indicated that men had a higher cortisol increase in response to the stressor compared to women, as seen in other studies. They also found that this increased stress response in men was positively correlated ($r = 0.61$) with the number of correctly recalled items, indicating enhanced memory recollection. Although women exhibited increases in cortisol in response to the stressor, there was no correlation with behavioral performance. Furthermore, there was no difference between stressed and control women in the number of items recalled (Andreano & Cahill, 2006). The findings of this study also suggest that men not only have a greater response to cortisol, but that this elevation of cortisol correlates with enhanced recollection of story details. Research with humans has shown instances in which men have enhanced memory for fear learning and recalling details of a story in response to an acute stressor compared to non-stressed men (Andreano & Cahill, 2006; Zorawski et al., 2006). Further studies reveal that an elevation in
cortisol is a key factor in influencing behavior (Cahill et al., 2003). Interestingly, this contradicts what studies have found with male rats exposed to chronic stress and tested on spatial memory.

Further studies assessed how induced stress was impacted by menstrual cycle phase. Andreano and colleagues (2008) examined how cortisol levels were affected in women across the menstrual cycle in response to the cold pressor task. They found that women in the mid-luteal phase had the greatest elevations in cortisol in response to the acute stressor compared to women in the early follicular or late follicular phases (Andreano et al., 2008). Similarly, another group found that women in the luteal phase revealed a negative correlation between cortisol level, in response to the TSST, and verbal memory performance on the RAVLT, such that as cortisol level increased, performance decreased for this group of women (Espin et al., 2013).

Others have examined how memory performance was affected in men, women in the early-follicular phase (low estradiol), and women in mid-cycle (high estradiol) in response to an acute stressor (Antov & Stockhorst, 2018). Participants underwent a stress task in which a speech was delivered to authority figures, similar to the TSST, however negative feedback was also directed back to them. They were then tested on a declarative memory task in which they were presented with a story and were asked to recall details either immediately, after a short delay (35 minutes), or after a long delay (24 hours). Women in the early-follicular phase recalled significantly fewer details compared to mid-cycle women and men at both the short and long-term delays for the stressed group. No group differences occurred between men and women in the control groups. These researchers suggested that women in the mid-cycle phase, during which estradiol levels are high, may be protected against any negative effects of pre-learning stress on declarative memory consolidation (Antov & Stockhorst, 2018). The findings of this study contradict what others have shown in which women in mid-cycle phases have a greater
stress response and then in turn have impaired performance in response to the stress (Andreano et al., 2008; Espin et al., 2013).

Overall, the studies examining the impact of stress on learning and memory indicate that results are dependent on length of stress (acute vs. chronic), type of task (radial arm maze, Morris water maze, object location, etc.), and sex (male vs. female). Although both rodents (Table 1) and humans (Table 2) were affected by stress, males and females displayed different patterns. In non-human studies, it seems that exposure to chronic stressors led to deficits in behavior for males on various tasks including the radial arm maze (Luine et al., 1994; 1996), Morris water maze (Kitraki et al., 2004), object location (Beck & Luine, 2002), and Y-maze (Conrad et al., 2003). In contrast, females had enhanced behavior in response to a chronic stressor on tasks like radial arm maze (Bowman et al., 2001), Morris water maze (Kitraki et al., 2004), and object location (Beck & Luine, 2002). Perhaps females are either adapting to stress better than males, or females have a reduced sensitivity to chronic stress exposure. In human studies, it has been shown that males typically have a greater response to cortisol (Andreano & Cahill, 2006; Guenzel et al., 2014; Mazurka et al., 2017; Zorawski et al., 2006), either with direct induction or in response to an acute stressor. Furthermore, this enhanced elevated cortisol response in men has led to better performance on fear acquisition learning (Zorawski et al., 2006) and in recalling details of a story (Andreano & Cahill, 2006). For women, an increased stress response did not correlate with behavioral performance (Andreano & Cahill, 2006; Zorawski et al., 2006). However, in examining estradiol and menstrual cycle phase, research has shown that women with high levels of estradiol show an increased cortisol response (Andreano et al., 2008; Espin et al., 2013). Based on previous findings, it seems prudent to examine how induction of stress impacts spatial memory performance in men and women. Prior work has
assessed the impact of stress on various types of learning and memory but more needs to be done in humans with spatial memory. Additionally, the results are often conflicting and not much research has focused on how estradiol levels may impact the relationship between stress and spatial memory. Different types of memory may yield different patterns of results. Furthermore, additional research could aim to clarify how pre-existing sex differences in specific types of memory are affected by sex hormones, anxiety, and stress. Below are the central aims and study descriptions including predictions and hypotheses.

**Table 1. Summary of Behavioral Experiments with Induced Stress in Non-humans**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Stressor</th>
<th>Behavioral Task</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male rats</td>
<td>Chronic restraint for 21 days</td>
<td>Radial arm maze</td>
<td>Stressed males had impaired performance compared to control males</td>
<td>Luine et al., 1994; 1996</td>
</tr>
<tr>
<td>Female rats</td>
<td>Chronic restraint for 21 days</td>
<td>Radial arm maze</td>
<td>Stressed females had enhanced performance compared to control females</td>
<td>Bowman et al., 2001</td>
</tr>
<tr>
<td>Male rats</td>
<td>Chronic restraint for 21 days</td>
<td>Object location</td>
<td>Single-housed males had impaired memory compared to pair-housed males, control males, single-housed females, and pair-housed females</td>
<td>Beck &amp; Luine, 2002</td>
</tr>
<tr>
<td>Single or pair housed</td>
<td>Chronic restraint for 21 days</td>
<td>Object location</td>
<td>Stressed females (single- or pair-housed) performed as well as control males and better than control females</td>
<td></td>
</tr>
<tr>
<td>Male rats</td>
<td>Chronic restraint for 21 days</td>
<td>Y-maze</td>
<td>Stressed males had impaired performance compared to control males</td>
<td>Conrad et al., 2003</td>
</tr>
<tr>
<td>Male rats</td>
<td>Chronic restraint for 21 days</td>
<td>Morris water maze</td>
<td>Stressed males had impaired performance compared to control males</td>
<td>Kitraki et al., 2004</td>
</tr>
<tr>
<td>Male rats</td>
<td>Tail shock – once a minute for 30 minutes</td>
<td>Fear conditioning</td>
<td>Stressed males had enhanced conditioning compared to controls</td>
<td>Shors, 2001</td>
</tr>
<tr>
<td>Female rats</td>
<td>Tail shock – once a minute for 30 minutes</td>
<td>Fear conditioning</td>
<td>Stressed females in proestrus had impaired conditioning compared to stressed females in estrus</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Control females in proestrus had enhanced conditioning compared to other control females</td>
<td>Shors, 1998</td>
</tr>
</tbody>
</table>
Table 2. Summary of Behavioral Experiments with Induced Stress in Humans

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Stressor</th>
<th>Behavioral Task</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men and women</td>
<td>10mg of cortisol or placebo</td>
<td>Declarative memory task</td>
<td>Those treated with cortisol had impaired performance on the declarative memory and spatial thinking tasks compared to those given placebo</td>
<td>Kirshbaum et al., 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spatial thinking task</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Procedural memory task</td>
<td>There was no difference between the cortisol or placebo treated groups on the procedural memory task</td>
<td></td>
</tr>
<tr>
<td>Adolescent boys and girls</td>
<td>TSST</td>
<td>N/A</td>
<td>Adolescent boys had a greater cortisol response to the TSST than girls</td>
<td>Mazurka et al., 2017</td>
</tr>
<tr>
<td>Men and women</td>
<td>CPT</td>
<td>N/A</td>
<td>Men had a greater cortisol response to the CPT than women</td>
<td>Guenzel et al., 2014</td>
</tr>
<tr>
<td>Men and women</td>
<td>Mild shock</td>
<td>Fear conditioning</td>
<td>Cortisol levels post-acquisition were positively correlated with conditioning in men but not women</td>
<td>Zorawski et al., 2006</td>
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<td></td>
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<td>Post-acquisition cortisol was also positively correlated with fear consolidation in men with a high cortisol response but not in men with a low cortisol response or in women</td>
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<tr>
<td>Men and women</td>
<td>CPT</td>
<td>Declarative memory task</td>
<td>Men had a greater cortisol response to the CPT than women</td>
<td>Andreano &amp; Cahill, 2006</td>
</tr>
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<td>Stressed men had enhanced memory recollection; cortisol response was positively correlated with memory</td>
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<td>Women had no correlation between stress response and behavior; there were no differences in behavior between stressed and control women</td>
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<tr>
<td>Women (menstrual cycle phases)</td>
<td>CPT</td>
<td>N/A</td>
<td>Women in the mid-luteal phase had a greater cortisol response than women in the early or late follicular phases</td>
<td>Andreano et al., 2008</td>
</tr>
<tr>
<td>Women (menstrual cycle phases)</td>
<td>TSST</td>
<td>RAVLT</td>
<td>Women in the luteal phase had a negative correlation between cortisol response and verbal memory</td>
<td>Espin et al., 2013</td>
</tr>
<tr>
<td>Men and women (menstrual cycle phases)</td>
<td>TSST</td>
<td>Declarative memory task</td>
<td>Women in the early follicular phase had impaired performance compared to women in mid-cycle and men</td>
<td>Antov &amp; Stockhorst, 2018</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>There was no sex difference in behavior for the controls</td>
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Note: TSST: Trier Social Stress Task; CPT: Cold Pressor Task; RAVLT: Rey Auditory Verbal Learning Test
1.8 Central Aims

The goal of these studies was to investigate multiple factors that influence hippocampal-dependent spatial memory. Because estradiol impacts hippocampal structure and function, the effects of estradiol level in women were assessed in all three studies. Additionally, the impact of general anxiety on behavior and memory was examined in both men and women. Furthermore, because the hippocampus is also susceptible to changes in response to stress, the role of induced stress and its relationship with estradiol and spatial memory was examined.

First, the impact of estradiol was considered as a potential facilitator in spatial memory performance. Specifically, how levels of estradiol in women contribute to their behavioral performance on multiple spatial memory domains was assessed. Women at the low and high estradiol phases of their natural menstrual cycle were compared to each other and to women who were taking oral contraceptives. Behavioral performance on a virtual version of the Morris water task, a virtual version of the radial arm maze, and a mental rotation task was examined. Second, to further assess how general anxiety levels impact learning and memory, performance on spatial and verbal memory tasks in both men and women was measured. Behavioral performance on the virtual Morris water task, a declarative verbal memory task, and a verbal fluency task were assessed. Third, to explore how induced stress impacts memory in men and women differentially, memory was assessed on multiple tasks. Stress was induced via a virtual version of the Trier social stress task. Behavior was measured on the Morris water task, an object location task, a spatial working memory task, and mental rotation task.

The results from each of the studies provide valuable information on how spatial memory is impacted by estradiol, anxiety, and stress. Specifically, the findings from these studies help to understand how men and women differ on a variety of memory tasks, which types of task they differ on, and potentially provide solutions for mitigating sex differences. Furthermore, how
women with varying levels of estradiol perform on behavioral tasks can determine whether learning and memory abilities change in response to changes in sex hormones. Knowing this information is useful for women in everyday life situations.

II. The Impact of Estradiol Levels on Spatial Memory in Women (Study 1)

2.1 Introduction

As prior research has shown, women with high levels of estradiol performed better than women with low levels of estradiol on tasks that examined spatial working memory (Bartholomeusz et al., 2008; Hampson & Morley, 2013), verbal memory (Hussain et al., 2016; Protopopescu et al., 2008; Solis-Ortiz & Corsi-Cabrera, 2008), and visuospatial memory (Solis-Ortiz & Corsi-Cabrera, 2008). However, some research has shown the converse relationship on tasks that measure mental rotation ability, in that women with low levels outperform women with high levels (Hausmann et al., 2000; Mantyla 2013; McCormick & Teillon, 2001). Given that there are multiple measures of spatial memory such as working memory, spatial navigation, and mental rotation, it is important to explore estradiol’s impact on spatial memory behavior. Specifically, it is vital to examine how women with high levels of estradiol differ from women with low levels of estradiol on spatial memory tasks. Furthermore, considering the widespread use of OCs, it is important to examine whether the effects on hippocampal function differ between endogenous and exogenous estradiol in women (Beltz et al., 2015; Gogos et al., 2014; Griksiene & Ruksenas, 2011; Mordecai et al., 2008; Rivera et al., 1999).

This study was designed to test hippocampal-dependent spatial memory in female undergraduates taking OCs or who were in the high or low estradiol phases of their menstrual cycle. To assess spatial memory, two translational hippocampal-dependent tasks were used: a virtual Morris water task (VMWT) and a virtual radial arm maze (RAM). Previous research has
shown that in humans, these tasks activate the hippocampus during fMRI (Astur et al., 2005; Shipman & Astur, 2008) and that damage to the hippocampus impairs performance on these tasks (Astur et al., 2005; Goodrich-Hunsaker & Hopkins, 2010). Interestingly, prior research has shown a sex difference (male advantage) on the VMWT but not on the RAM, indicating that these two spatial tasks tap into different mechanisms of spatial memory (Astur et al., 2004). Furthermore, due to the findings demonstrating that estradiol facilitates memory consolidation in female rodents (Boulware et al., 2013; Fernandez et al., 2008; Frankfurt & Luine, 2015; Kim et al., 2016), testing was conducted over two consecutive days in order to examine the impact of estradiol on memory consolidation. A paper-and-pencil mental rotation task (MRT) was also used for further assessment of spatial abilities.

Based on studies showing that high levels of estradiol facilitates spatial navigation performance in rodents (Chow et al., 2013; Luine et al., 1998; Packard, 1998; Sandstrom & Williams, 2004), it was hypothesized that women with high levels of estradiol would outperform women with low levels of estradiol and women on OCs on the VMWT and the RAM, particularly at the beginning of the day two, which would reflect estradiol’s role in memory consolidation in humans. Additionally, given the results in previous research that has shown that women with low levels of estradiol perform better than women with high levels on this task (Hausmann et al., 2000; Mantyla 2013; McCormick & Teillon, 2001), it was hypothesized that women with low levels of estradiol would perform the best on the MRT.

2.2 Method

2.2.1 Participants. A total of 251 female undergraduates ($M = 19.03$ years, $SD = 1.05$) were recruited from the University of Connecticut. Subjects received class credit for their participation. Some participants were excluded due to cybersickness, irregular menstrual cycles,
use of intrauterine devices (IUDs), or incomplete data sets, leaving 162 participants for analysis. For the 66 naturally cycling participants, the upper 40% of estradiol levels were used to create the high estradiol (HE) group (mean level = 3.89 pg/mL) and the lower 40% of estradiol levels yielded the low estradiol (LE) group (mean level = 1.19 pg/mL). The 40% cutoffs were used to generate two distinct groups and separate those individuals whose levels fell right in the middle. Of the 86 participants on OCs at the time of testing, 32 were on a monophasic type with an ethinylestradiol level that ranged between 0.020-0.035 mg and the same type of progestin (norethindrone) that ranged between 1.00-1.25 mg. Common OCs that met these criteria included Gildess Fe, Junel Fe, Lo Loestrin Fe, Lomedia 24 Fe, Minastrin 24, Alyacen, Zovia, and Microgestin. These users were chosen for a homogenous comparison. The remaining OC users were excluded given that their ethinylestradiol levels ranged from 0.01 to 0.05 mg and progestin levels varied from 0.1 to 3.0 mg. Additionally, many of these OCs were either biphasic or triphasic in which two or three different doses of the hormones were consumed across the menstrual cycle. The final groups included the HE group (N=27), LE group (N =27), and OC group (N =32).

2.2.2 Procedure. On day one, participants completed a general questionnaire after consent was obtained, and then completed the VMWT, RAM, and MRT. On day two, participants completed a retest on the VMWT and RAM. After completion of the tasks on day two, saliva was collected.

2.2.3 Virtual Morris Water Task. Participants were informed that they would find themselves in a virtual pool (Figure 1A), and that their goal was to escape as quickly as possible by navigating to a hidden goal platform. Initially there were four visible platform trials to control for any differences in visual, sensory, or motor behaviors. Trials started from four locations
Figure 1. Pictured above are third-person views of the virtual Morris water task used in Studies 1-3 (A) and virtual radial arm maze used in Study 1 (B). Salient cues are present throughout the room to distinguish each wall. Participants were in first person view during all tasks.

(north, south, east, and west) three times each for a total of twelve trials with a three second inter-trial interval (ITI) during which a blank screen appeared. A probe trial was then given in which the platform was removed, and the participant, unaware of this, was allowed to search for 20 seconds. On day two, participants received a probe trial first to test for memory retention of the previous day, followed by eight hidden platform trials and one final probe trial.

2.2.4 Virtual Radial Arm Maze. Participants were informed that they would find themselves in a virtual room that had eight runways extending out from a round middle area (Figure 1B). They were instructed to find rewards at the end of four arms as quickly as possible. They were told that only four of the eight arms contained a reward. Five trials were administered per day with an ITI of three seconds during which a blank screen appeared. A reference memory error was scored if the participant entered into an arm that was never rewarded. A working memory error was scored if the participant entered an arm that was previously entered during that trial, regardless of whether that arm was rewarded. Reference and working memory errors were not scored during trial 1 since the task was still being learned.

2.2.5 Mental Rotation Task. A pen and
paper mental rotation task adapted from Vandenberg & Kuse (1978) was used. Participants were shown a target object and two choices that matched the target but were rotated and two choices that did not match. Participants were to select both choices that matched. Participants were given four minutes to complete twelve problems. The number of correct responses was summed for a total score (maximum possible score: 24).

2.2.6 Saliva Collection. For naturally cycling participants, saliva was collected in individually marked 2 ml test tubes via a passive drool method and stored in a freezer and kept at -25°C until shipped. Saliva was collected once at the end of the session on day two. Samples were packed in dry ice and shipped to Salimetrics (State College, PA) and assayed for estradiol levels using a highly sensitive enzyme immunoassay. All estradiol assays were performed in duplicate and the average value was used as the participants’ value.

2.2.7 Analyses. For the virtual Morris water task on day one, a 3 (Block) by 3 (Group) ANOVA was conducted to examine participants’ distance to find the platform, with Block encompassing 4 consecutive trials in which participants started from each of the four locations. Distance was chosen as the dependent variable rather than time to control for any differences in motor speed with the joystick. A one-way ANOVA for Group was also conducted to measure participants’ percent of distance in the correct quadrant on the probe trial. For between group comparisons, Fisher’s LSD post-hoc analyses were conducted. On day two, a one-way ANOVA for Group was analyzed for the first block as a measure of memory consolidation. Furthermore, to directly compare the low and high estradiol groups on memory consolidation, a t-test was conducted for the first block of day two. A 3 (Block) by 3 (Group) ANOVA was also conducted to examine participants’ distance to find the platform. Finally, a one-way ANOVA for Group was also conducted to measure participants’ percent of distance in the correct quadrant on the
probe trial. For between group comparisons, Fisher’s LSD post-hoc analyses were conducted.

For the radial arm maze on day one, a 5 (Trial) by 3 (Group) ANOVA was conducted to examine distance across trials. As for the VMWT, distance was analyzed instead of time since it would not be confounded by differences in speed. A 5 (Trial) by 3 (Group) ANOVA was also conducted for working memory errors and for reference memory errors. On day two, a 5 (Trial) by 3 (Group) ANOVA was conducted to examine distance across trials. A 5 (Trial) by 3 (Group) ANOVA was also conducted for working memory errors and for reference memory errors.

For the mental rotation task, a one-way ANOVA for Group was conducted to analyze group differences in overall score on the task.

2.3 Results

2.3.1 Virtual Morris Water Task. On day one, there was a significant effect of Block, \(F(2, 82) = 14.18, p < 0.001\) (Figure 2), such that participant’s distance to find the platform was reduced across Blocks. However, the main effect of Group was not significant, \(F(2, 83) = 0.75,\)

![Image](image_url)

**Figure 2.** Mean distance to platform on the virtual Morris water task across days. Shorter distances indicate more direct paths (better performance). In Block 4, the high estradiol group exhibited significantly shorter distances to the platform than the low estradiol group \((p = 0.01)\). The high estradiol and contraceptive groups did not differ on any blocks across days.
non-significant (n.s.), nor was the Group by Block interaction, $F(2, 166) = 1.24$, n.s. Together, these data suggest hormone status did not affect task acquisition on day one.

On day two, there was no longer an effect of Block, $F(1, 83) = 1.58$, n.s., likely indicating that participants were at ceiling performance. As seen on day one, there also was no effect of Group, $F(2, 83) = 1.61$, n.s., nor a Group by Block interaction, $F(2, 83) = 0.69$, n.s. However, in an *a priori* analysis to compare the two naturally cycling groups on performance on the first block of testing on day two as a measure of retention, it was observed that the HE group traveled significantly less distance to find the platform relative to the LE group during the first block, $t(52) = 1.74$, $p = 0.01$, (Figure 2).

There was no effect of Group for the probe trial at the end of day one, $F(2, 135) = 0.36$, n.s., or at the end of day two, $F(2, 137) = 0.15$, n.s. for all three groups (Figure 3). At the start of day two, participants immediately performed a probe trial to assess memory of the previous day’s training. On this probe trial, there was a significant effect of Group, $F(2, 137) = 3.69$, $p = 0.01$, (Figure 2).

Figure 3. Percent of total distance in the quadrant that contained the platform during hidden trials for all probe trials on the virtual Morris water task. During the first probe on day two, the low estradiol group had a significantly lower percentage in the correct quadrant compared to the high estradiol group ($p = 0.01$) and the contraceptive group ($p = 0.04$).
Post-hoc tests revealed that the HE group had a significantly greater distance in the correct quadrant compared to the LE group (42% vs. 29% respectively), \( p = 0.01 \) (Figure 3). The OC group also had a significantly greater distance (38%) in the correct quadrant compared to the LE group, \( p = 0.04 \) (Figure 3).

### 2.3.2 Radial Arm Maze

There was a significant effect of Trial on day one indicating that the distance, \( F(4, 75) = 47.63, p < 0.001 \), number of working memory errors, \( F(4, 76) = 8.44, p < 0.001 \), and number of reference memory errors, \( F(4, 76) = 10.81, p < 0.001 \), decreased with training across trials (Figure 4). The same was

\[ \text{Distance Traveled Across Trials} \]

\[ \text{Working Memory Errors Across Trials} \]

\[ \text{Reference Memory Errors Across Trials} \]

**Figure 4.** Mean distance, number of working memory errors, and number of reference memory errors across trials on day one of the RAM. Shorter distances and lower errors scores indicate better performance. Error bars indicate standard error of the mean (SEM). There was a significant main effect of Trial for all measures on day one, in that distance \( (p < 0.001) \), number of working memory errors \( (p < 0.001) \), and number of reference memory errors \( (p < 0.001) \), decreased with training.
true for distance, $F(4, 65) = 13.98, p < 0.001$, and number of reference memory errors, $F(4, 65) = 7.03, p < 0.001$, on day two (Figure 5). There was no effect of Trial on day two for number of working memory errors for the HE ($M = 0.17, SD = 0.34$), LE ($M = 0.06, SD = 0.16$), and OC ($M = 0.35, SD = 0.77$) groups, likely due to a floor effect (Figure 5). There was no effect of Group on day one, $F(2, 78) = 1.10$, n.s., or day two, $F(2, 117) = 0.30, p > 0.1$, nor a Trial by Group interaction on day one, $F(2, 312) = 0.12$, n.s., or day two, $F(2, 272) = 0.70$, n.s.

2.3.3 Mental Rotation Task. The main effect of Group was not significant, $F(2, 83) = 0.93$, n.s., indicating no

![Distance Traveled Across Trials](image1)

![Working Memory Errors Across Trials](image2)

![Reference Memory Errors Across Trials](image3)

Figure 5. Mean distance, number of working memory errors, and number of reference memory errors across trials on day two of the RAM. Shorter distances and lower errors scores indicate better performance. Error bars indicate standard error of the mean (SEM). There was a significant main effect of Trial for distance ($p < 0.001$) and number of reference memory errors ($p < 0.001$), in that both decreased with training on day two. The effect of Trial was not significant for working memory errors on day two ($p > 0.05$).
differences in mental rotation abilities among the HE \((M = 10.89, SD = 3.56)\), LE \((M = 9.63, SD = 2.87)\), and OC \((M = 10.41, SD = 3.72)\) groups.

2.4 Conclusions

The results of this study showed that women with low estradiol levels had impaired performance on the virtual Morris water task at the start of day two, indicated by greater distances to find the platform and lower percentages of distance in the target quadrant during the probe trial. These findings suggest that low endogenous levels of estradiol hindered memory consolidation relative to high endogenous levels or exogenous synthetic levels of estradiol. The lack of performance differences on the VMWT among all three groups on day one indicates that all women could successfully acquire the task, and that estradiol levels may not be critical for the acquisition of spatial information in women. Conversely, the results of the VMWT indicated better memory consolidation for the HE group compared to the LE group at the start of day two for both distance to platform and probe trial performance.

There were no group differences on the RAM in analyses of distance, reference memory errors, or working memory errors on day one or two indicating that all groups were able to successfully learn and retain the task. This supports our lab’s previous research that shows the two virtual tasks measure different aspects of spatial memory (Astur et al., 1998; Astur et al., 2004), and that estradiol level influences may be specific to more complex spatial memory tasks such as the VMWT. This task is more ambiguous, in that that there are no direct paths to the goal location and participants are free to move in any direction. In contrast, the RAM forces the participants to navigation within specific arms which could make it easier to complete.

The results yielded no significant group differences on the mental rotation task. Men typically outperform women on the MRT (Astur et al., 2004; Halpern, 2013; Linn & Petersen,
1985). Furthermore, as seen in previous studies, women in phases with low estradiol levels typically perform better than women in phases with high estradiol levels (Hausmann et al., 2000; Mantyla 2013; McCormick & Teillon, 2001). Although this result was expected in the current study, no group differences were observed. Other studies have also found no difference in performance on the MRT between OC users and naturally cycling women (Mordecai et al., 2008; Rosenberg & Park, 2002). Thus, it is likely that this task may be less sensitive to changes in estradiol level in women. Given that the mean scores for all three groups ranged from around 9.6 to 11 out a potential score of 24, it is likely that the task was equally as difficult for all groups. Perhaps if task difficulty was decreased or the time limit to complete questions was lengthened, group differences may have been more apparent.

Finally, no major deficit was seen in the OC group indicating that this group performed just as well as the naturally cycling groups. The findings of this study may have been impacted by not recruiting participants in particular menstrual cycle phases. While this method provided us with a full range of estradiol levels, by including women in the luteal phase of their cycle, progesterone levels may have influenced behavior in these tasks.

### III. How General Anxiety Levels Impact Sex Differences in Spatial Memory (Study 2)

#### 3.1 Introduction

As prior work has shown, anxiety has resulted in impaired verbal memory performance (Ikeda et al., 1996) and spatial memory performance (Walf et al., 2009). Additionally, it has been established that men perform better on spatial navigation tasks than women (Astur et al., 1998; Galea & Kimura, 1993; Moffat et al., 1998; Postma et al., 2004; Sandstrom et al., 1998). In bridging a link between anxiety and sex differences in spatial memory, research has observed that women have elevated spatial anxiety on spatial navigation tasks which is associated with
different strategy use than men and may have negative implications on their behavior (Lawton, 1994; Schmitz, 1999). Some believe that anxiety impacts spatial memory via hippocampal processes (Gray & McNaughton, 2000). Furthermore, individuals who suffer from anxiety have more difficulty in forming new memories, have impaired learning, and have deficits in memory consolidation (Maeng & Milad, 2016).

Given that women have an increased risk for an anxiety disorder (Altemus et al., 2014; Kessler et al., 2005; Maeng & Milad, 2016; Newhouse & Albert, 2015), have twice the lifetime rate of the disorder (Altemus et al., 2014), and experience symptoms of the disorder to a higher severity than men (Altemus et al., 2014; Maeng & Milad, 2016), it is important to understand how general anxiety levels impacts learning and memory. Specifically, it is vital to examine how men and women are impacted by general anxiety on many types of memory including verbal and spatial based on prior work that show both are affected.

Prior research has indicated that hormone levels can impact spatial memory performance in females. OVX rats that received immediate estradiol treatment had reductions in anxiety and improvements on an object placement task compared to OVX rats without hormonal treatment (Walff et al., 2009). In human studies, on a spatial working memory task, Hampson & Morley (2013) found no sex differences between number of errors women with high levels of estradiol and men produced in a delay condition. However, they found that women with low levels of estradiol produced the highest number of errors across all trials compared to women with high levels of estradiol, indicating poorer performance (Hampson & Morley, 2013). Although men may have an advantage on certain spatial memory tasks, estradiol may play a role in how women are able to perform on those spatial tasks. Additionally, high spatial anxiety may differentially impact memory performance in women more so than men (Lawton, 1994; Schmitz, 1999).
Furthermore, research has shown that women typically perform better on verbal tasks such as recognition, recollection, and fluency compared to men (Kramer et al., 1988; Lewin et al., 2001; Munro et al., 2012; Weiss et al., 2003; 2006). Kramer and colleagues (1988) examined sex differences on the California Verbal Learning Test (CVLT). After experimenters read aloud a list of words, participants were to recall as many words as possible. Five trials were administered followed by a distractor list and recollection was tested again. Following a 20-minute delay, recollection was tested once more followed by a recognition test. Type of learning strategy and errors (incorrect words recalled or recognized) were also assessed. Results indicated that women had better free recollection for both the immediate trials and the delayed condition, compared to men. Women also tended to use a semantic clustering technique in which they actively organized and grouped words with similar meanings together for better recollection. Men were more likely to group words in serial order (order in which words were presented). There were no differences in recognition or in the number of errors between sexes (Kramer et al., 1988). The findings of this study suggest that women had better verbal recollection than men and that superior performance was likely due to differences in strategy use.

Another study tested men and women on three types of verbal memory tasks (Weiss et al., 2003). Verbal recognition was assessed on the Warrington Recognition Memory Test for Words in which participants are presented with 50 words and then shown pairs of words (a target and a novel word) from which they are to choose the word they were previously exposed to (force choice). Verbal recollection was tested on the Rivermead Behavioral Memory Test in which participants listen to a short story that is read aloud and are required to write down as much of the story that was retained immediately after and after a delay. Verbal fluency was examined by instructing participants to generate as many words as possible with specific letters.
Results indicated that women had better performance on the Warrington recognition task by identifying more correct words than men. Women also had superior performance on the verbal fluency task in which they generated more words per letter than men. However, there were no sex differences in recall of story details at the immediate and delayed conditions (Weiss et al., 2003). Others have also found that women have better performance on verbal fluency compared to men (Lewin et al., 2001; Weiss et al., 2006).

Another group also tested verbal fluency, recollection, and recognition in men and women (Munro et al., 2012). On the verbal fluency task, participants were to generate as many words as possible that begin with specific letters. The Hopkins Verbal Learning Test was also administered. This task is similar to the CVLT and RAVLT but much shorter. Twelve words were read aloud (4 words from each of 3 semantic categories) for three trials. Immediately after each trial, participants recalled as many words as possible. Following these trials, participants were presented with a list of 24 words (12 old and 12 new) and were asked to identify whether each word was part of the initial list or not. Women generated more words on the verbal fluency task compared to men. Additionally, women had better recollection during the three trials of the HVLT and better word discrimination during the recognition trial, compared to men (Munro et al., 2012). Furthermore, as discussed, studies have shown that women in the ovulatory group of their cycle, produced fewer words on a verbal fluency task compared to women in the late-luteal phase of their cycle (Solis-Ortiz & Corsi-Cabrera, 2008). However, not much research has focused on how hormone levels impact the female advantage on verbal tasks.

This study was designed to test the impact of general anxiety on hippocampal-dependent spatial memory and verbal memory in undergraduates. In order to assess general anxiety, the State-Trait Anxiety Inventory (STAI), which measures both state and trait anxiety (Spielberger et
al., 1983) was used. Trait anxiety consists of feelings of apprehension, tension, and worry that can coincide with increased activity of the autonomic nervous system (Spielberger, 2013). State anxiety is an emotional state that can fluctuate in response to stressors that an individual experiences (Spielberger, 2013). This survey evaluates subjective feelings of nervousness, worrying, apprehension, calmness, confidence, security, and activation of the autonomic nervous system (Julian, 2011; Spielberger et al., 1983). Scores on the STAI were used to determine the anxious and non-anxious groups. Spatial memory was assessed on the VMWT whereas, verbal memory was tested on the Hopkins Verbal Learning Test (HVLT) and a verbal fluency task. The VMWT was chosen as the test for spatial memory since it reveals a male advantage on the task (Astur et al., 1998; Astur et al., 2004). The HVLT was chosen to measure both recognition and recollection of verbal memory because of its brevity and based on prior research that has shown sex differences (Munro et al., 2012). A verbal fluency task was chosen as a secondary measure of verbal memory that encompasses a different procedure: the spontaneous production of words for specific letters. As prior research has shown, women typically perform better on verbal fluency (Lewin et al., 2001; Munro et al., 2012; Weiss et al., 2003; 2006).

Performance was compared among women at the naturally low and high estradiol phases of their cycle, women on OCs, and men. It was hypothesized that men will outperform women on the VMWT for both distance to platform and probe trial performance, and the reverse relationship would be seen on verbal recollection, recognition, and fluency. Additionally, it was hypothesized that anxious men will have impaired spatial and verbal memory performance compared to non-anxious men. It was predicted that anxious women in general will have impaired performance on both spatial and verbal memory compared to non-anxious women. Additionally, it was also hypothesized that anxious women in the low estradiol phase will exhibit
impairments on behavioral performance compared to anxious women in the high estradiol phase.

3.2 Method

3.2.1 Participants. A total of 179 undergraduates ($M = 18.78$ years, $SD = 1.07$) were recruited from the University of Connecticut. Subjects received class credit for their participation. Out of the participants that initially consented, 35 participants were excluded for various reasons (cybersickness, irregular menstrual cycles, use of IUDs, or incomplete data sets). Of the remaining 144 participants, 105 were females.

Based on data obtained by the State-Trait Anxiety Inventory (STAI), men and women were categorized into anxious and non-anxious groups. Those that scored an 80 (a combination score of both state and trait anxiety) or above were categorized as anxious, which is a standard procedure (Julian, 2011). The results yielded 20 anxious men and 45 anxious women. The remaining 19 men and 60 women were characterized as non-anxious/normal control (Table 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>Women</th>
<th>Men</th>
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<td>Anxious</td>
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<tr>
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<tr>
<td>Non-ovulatory</td>
<td>17</td>
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<td>Contraceptives</td>
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<tr>
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<td></td>
</tr>
<tr>
<td>Contraceptives</td>
<td>28</td>
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</tr>
</tbody>
</table>

Women were also further categorized by hormone group based on menstrual cycle information. The date of last menstruation was subtracted from the study date to calculate the approximate menstrual cycle phase of each participant. With the start of menstruation characterized as day 0, those that were between days 0 to 8 or within days 25 to 35 were
considered to be in the non-ovulatory phase. These women would have low estradiol levels given that the day ranges include those in the late-luteal and early follicular phases. Those that were between days 10 to 17 were considered to be in the ovulatory phase. These women would have high estradiol levels which is indicative of the late-follicular and ovulatory phases. Women between any of the remaining days were excluded due to likely having medium levels of estradiol. Women who had cycles longer than 35 days were excluded from the study as they were considered to have irregular cycles. The cut-offs for each group were based on prior work that show reliable methods of calculating menstrual cycle phases (Mihm et al., 2011).

Of the 45 anxious women, 18 were on OCs at the time of testing, 10 were in the ovulatory phase of their cycle, and 17 were in the non-ovulatory phase of their cycle. Of the 60 normal women, 28 women were on OCs at the time of testing, 10 were in the ovulatory phase of their cycle, and 22 were in the non-ovulatory phase of their cycle (Table 3). For OC users, women had to be on a specific type to be eligible for the study. Only monophasic OCs with an ethinylestradiol level that ranged between 0.020-0.035 mg and the same type of progestin (norethindrone) that ranged between 1.00-1.25 mg, were included. None of the women were pregnant at the time of testing.

3.2.2 Procedure. After receiving informed consent, participants answered a general questionnaire. Following this, participants completed a set of assessments: the State-Trait Anxiety Inventory (STAI) which measures both state and trait anxiety (Spielberger et al., 1983); the Positive and Negative Affect Schedule (PANAS) which measures the level of positive and negative affect (Watson, Clark, & Tellegen, 1988); and The Profile of Mood States (POMS): which evaluates a variety of mood states including tension, depression, anger, vigor, fatigue, and confusion (McNair & Droppleman, 1971). The PANAS and POMS were added as additional
subjective measures to control for any differences in mood or feelings. Participants then completed the HVLT, the VMWT, and a verbal fluency task.

3.2.3 Hopkins Verbal Learning Test. This task measures immediate recall and recognition of words, which has been shown to have a female advantage (Munro et al., 2012). A list of twelve familiar words from three semantic categories (professions, sports, and food) was read aloud three times. One word was presented every two seconds. Participants had to recall as many words as possible after each presentation of the list. Following this, participants are read a list of 24 words, half were from the original list, half were new words. Participants had to state whether each word was from the original list or a new word. For recollection trials, the total number of correctly recalled words was summed for all three trials for an overall score. For recognition, the number of incorrect words was subtracted from the number of correct words for an overall score. A discrimination index was also calculated based on both recollection and recognition.

3.2.4 Virtual Morris Water Task. Participants completed the same task that was previously described for day one of Study 1 (the second part on day two from Study 1 was not included). The task began with four visible platform trials followed by twelve hidden platform trials and one probe trial.

3.2.5 Verbal Fluency. This task measures spontaneous production of words and in some cases has shown a female advantage (Lewin et al., 2001; Munro et al., 2012; Weiss et al., 2003; 2006), and in some cases has shown no sex differences (Wallentin, 2009). Participants were given three letters (F, A, and S) and were instructed to generate as many words as possible that began with that letter, excluding proper nouns and variations of the same word. One letter was
presented at a time for one minute. The number of words (minus exclusions) was summed for each letter. The total sum per letter were then added for a total task score.

3.2.6 Analyses. For the virtual Morris water task, a 3 (Block) by 2 (Anxiety) by 2 (Sex) ANOVA was conducted to examine participants’ distance to find the platform. A 2 (Anxiety) by 2 (Sex) ANOVA was conducted on the probe trial to test for differences in percent of participants’ distance in the correct quadrant. Next, a 3 (Block) by 2 (Anxiety) by 4 (Group: men, ovulatory women, non-ovulatory women, and women on OCs) ANOVA was conducted to examine participants’ distance to find the platform. A 2 (Anxiety) by 4 (Group) ANOVA was conducted on the probe trial to test for differences in percent of participants’ distance in the correct quadrant. For any between group comparisons, Fisher’s LSD post-hoc analyses were conducted.

For the Hopkins verbal learning test, a 2 (Anxiety) by 2 (Sex) ANOVA was conducted to compare overall score on the task for recollection. Next, a 2 (Anxiety) by 4 (Group) ANOVA was conducted to compare overall score on the task for recollection. A 2 (Anxiety) by 2 (Sex) ANOVA was conducted to compare overall score on the task for recognition. Additionally, 2 (Anxiety) by 4 (Group) ANOVA was conducted to compare overall score on the task for recognition. For any between group comparisons, Fisher’s LSD post-hoc analyses were conducted.

For verbal fluency, a 2 (Anxiety) by 2 (Sex) ANOVA was conducted to compare overall score on the task. Next, a 2 (Anxiety) by 4 (Group) ANOVA was conducted to compare overall score on the task. For between group comparisons, Fisher’s LSD post-hoc analyses were conducted.

3.3. Results

3.3.1 Virtual Morris Water Task. There was a significant effect of Block, $F(2, 139) = 53.18, p < 0.001$, such that participant’s distance to find the platform was reduced across Blocks. However, results indicated no main effect of Sex, $F(1, 140) = 0.41, n.s.,$ no main effect of
Anxiety, $F(1, 140) = 0.96$, n.s., and no Sex by Anxiety interaction, $F(1, 140) = 0.18$, n.s., on the distance across blocks (Figure 6). In analyzing Group instead of Sex for distance across blocks, there was a significant effect of Block, $F(2, 135) = 55.45, p < 0.001$, such that participant’s distance to find the platform was reduced across blocks. However, results indicated no main effect of Group, $F(3, 136) = 0.15$, n.s., no main effect of Anxiety, $F(1, 136) = 1.53$, n.s., and no Group by Anxiety interaction, $F(3, 136) = 1.45$, n.s., on the distance across blocks (Figure 7).

![Distance to Platform on the VMWT by Sex](image)

**Figure 6.** Mean distance to platform on the virtual Morris water task across blocks. Shorter distances indicate more direct paths (better performance). There was no main effect of Sex or Anxiety nor an interaction across blocks.

For probe trial performance, there was a significant main effect of Sex, $F(1, 140) = 3.96$, $p = 0.05$, however there was no main effect of Anxiety, $F(1, 140) = 0.24$, n.s, or a Sex by Anxiety interaction, $F(1, 140) = 1.70$, n.s (Figure 8). As expected, men had a significantly greater percent of distance (53%) in the quadrant that previously held the platform compared to women (45%), $F(1, 142) = 4.13, p = 0.04$. In analyzing Group instead of Sex, there was no main
effect of Group, \( F(3, 136) = 1.35, \) n.s., no main effect of Anxiety, \( F(1, 136) = 0.09, \) n.s., and no Group by Anxiety interaction, \( F(3, 136) = 1.13, \) n.s (Figure 9).

### 3.3.2 Hopkins Verbal Learning Test

For recollection on the HVLT (Table 4), there was no main effect of Sex, \( F(1, 193) = 1.09, \) n.s., no main effect of Anxiety, \( F(1, 193) = 0.10, \) n.s., nor an interaction, \( F(1, 193) = 1.08, \) n.s. There was no effect of Group, \( F(3, 189) = 0.43, \) n.s., no main effect of Anxiety, \( F(1, 189) = 1.40, \) n.s., nor an interaction, \( F(3, 189) = 0.61, \) n.s.

For recognition on the HVLT (Table 4), there was no main effect of Sex, \( F(1, 192) = 2.22, \) n.s., no main effect of Anxiety, \( F(1, 192) = 0.18, \) n.s., nor an interaction, \( F(1, 192) = 0.45, \) n.s. There was no main effect of Group, \( F(3, 188) = 0.73, \) n.s., no main effect of Anxiety, \( F(1, 188) = 0.01, \) n.s., nor an interaction, \( F(3, 188) = 0.25, \) n.s.

![Distance to Platform on the VMWT by Group](image)

**Figure 7.** Mean distance to platform on the virtual Morris water task across blocks. Shorter distances indicate more direct paths (better performance). There was no main effect of Group or Anxiety nor an interaction across blocks (Anx: anxious, Norm: not-anxious).
3.3.3. **Verbal Fluency.** There was no main effect of Sex, $F(1, 193) = 1.00$, n.s., no main effect of Anxiety, $F(1, 193) = 0.52$, n.s., nor an interaction, $F(1, 193) = 0.01$, n.s (Table 4). There was no main effect of Group, $F(3,189) = 0.58$, n.s., no main effect of Anxiety, $F(1, 189) = 0.09$, n.s., nor an interaction, $F(3, 189) = 0.98$, n.s (Table 4).

3.3.4 **Positive and Negative Affect Schedule (PANAS).** There was a main effect of Anxiety for the positive affect questions on the PANAS, $F(1,192) = 21.08$, $p < 0.001$, in that the non-anxious participants had higher positive affect than the anxious participants (Table 5). There was no effect of Sex, $F(1, 192) = 2.18$, n.s., nor an interaction, $F(1, 192) = 0.12$, n.s. In analyzing Group instead of Sex, there was a main effect of Anxiety for the positive affect questions on the PANAS, $F(1,188) = 22.96$, $p < 0.001$, in that the non-anxious participants had higher positive affect than the anxious participants (Table 5). There was no effect of Group, $F(3, 188) = 1.82$, n.s., nor an interaction, $F(3, 188) = 0.70$, n.s.

There was a main effect of Anxiety on the negative affect questions on the PANAS as well, $F(1,192) = 55.59$, $p < 0.001$, in that the anxious participants had significantly higher negative affect than the non-anxious participants (Table 5).

---

**Figure 8.** Percent of total distance in the quadrant that contained the platform during hidden trials for the probe trial on the virtual Morris water task, based on anxiety levels (Anx: anxious, Norm: not-anxious). Men had a significantly greater percentage in the correct quadrant compared to women ($p = 0.04$).
participants had higher negative affect than the non-anxious participants (Table 5). There was no effect of Sex, $F(1, 192) = 0.47$, n.s., nor an interaction, $F(1, 192) = 0.52$, n.s. In analyzing Group instead of Sex, there was a main effect of Anxiety for the positive affect questions on the PANAS, $F(1, 188) = 60.44$, $p < 0.001$, in that the non-anxious participants had higher positive affect than the anxious participants (Table 5). There was no effect of Group, $F(3, 188) = 0.59$, n.s., nor an interaction, $F(3, 188) = 0.26$, n.s.

3.3.5 Profile of Mood States (POMS). There was a main effect of Anxiety for the POMS, $F(1,193) = 41.44$, $p < 0.001$, in that anxious group showed higher scores for tension, depression, anger, vigor, fatigue, and confusion than the non-anxious group (Table 5). There was no effect of Sex, $F(1, 193) = 0.24$, n.s., nor an interaction, $F(1, 193) = 2.17$, n.s. In analyzing

![Percent of Total Distance in the Correct Quadrant by Group](image)

**Figure 9.** Percent of total distance in the quadrant that contained the platform during hidden trials for the probe trial on the virtual Morris water task, based on anxiety levels (Anx: anxious, Norm: not-anxious). There was no main effect of Group or Anxiety nor an interaction.
Group instead of Sex, there was a main effect of Anxiety for the POMS, $F(1,189) = 31.67$, $p < 0.001$, in that the anxious group showed higher scores for tension, depression, anger, vigor, fatigue, and confusion than the non-anxious group (Table 5). There was no effect of Group, $F(3, 189) = 1.82$, n.s., nor an interaction, $F(3, 189) = 2.93$, n.s.

3.3.6 Mood and Behavior. The PANAS-Positive, PANAS-Negative, and POMS scores did not yield any correlations with performance on the VMWT (distance and probe trial), HVLT, or verbal fluency, $p$’s > 0.5 (Table 6).

<table>
<thead>
<tr>
<th>Table 4. Means and Standard Deviations for Verbal Tasks</th>
</tr>
</thead>
<tbody>
<tr>
<td>HVLT Recollection</td>
</tr>
<tr>
<td>Mean</td>
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<tr>
<td>-------</td>
</tr>
<tr>
<td>Anx</td>
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<td>Norm</td>
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<tr>
<td>Women</td>
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<tr>
<td>Men</td>
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<tr>
<td>Women Anx</td>
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<tr>
<td>Women Norm</td>
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<tr>
<td>Men Anx</td>
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<tr>
<td>Men Norm</td>
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<tr>
<td>Ovulatory</td>
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<tr>
<td>Non-Ovul</td>
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<tr>
<td>OCs</td>
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<td>Non-Ovul Anx</td>
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<tr>
<td>Non-Ovul Norm</td>
</tr>
<tr>
<td>OCs Anx</td>
</tr>
<tr>
<td>OCs Norm</td>
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</table>

Table 5. Means and Standard Deviations for Mood Assessments

<table>
<thead>
<tr>
<th></th>
<th>STAI</th>
<th>POMS</th>
<th>PANAS-Positive</th>
<th>PANAS-Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Anx</td>
<td>97.98</td>
<td>14.04</td>
<td>113.54*</td>
<td>18.75</td>
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<tr>
<td>Norm</td>
<td>64.77</td>
<td>9.37</td>
<td>98.38</td>
<td>13.41</td>
</tr>
<tr>
<td>Women</td>
<td>78.78</td>
<td>20.62</td>
<td>105.19</td>
<td>17.51</td>
</tr>
<tr>
<td>Men</td>
<td>80.06</td>
<td>18.51</td>
<td>103.81</td>
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<tr>
<td>Women Anx</td>
<td>98.33</td>
<td>14.49</td>
<td>112.89</td>
<td>19.60</td>
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<tr>
<td>Women Norm</td>
<td>64.26</td>
<td>9.69</td>
<td>99.59</td>
<td>13.36</td>
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<tr>
<td>Men Anx</td>
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<td>12.86</td>
<td>115.52</td>
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<tr>
<td>Men Norm</td>
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<tr>
<td>Ovulatory</td>
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<td>101.88</td>
<td>12.37</td>
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<td>Non-Ovul</td>
<td>77.37</td>
<td>19.62</td>
<td>106.40</td>
<td>19.43</td>
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<tr>
<td>Ovulatory Anx</td>
<td>95.62</td>
<td>18.07</td>
<td>101.38</td>
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<tr>
<td>Ovulatory Norm</td>
<td>68.62</td>
<td>6.27</td>
<td>102.38</td>
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<tr>
<td>Non-Ovul Anx</td>
<td>96.69</td>
<td>11.62</td>
<td>118.85</td>
<td>20.89</td>
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<tr>
<td>Non-Ovul Norm</td>
<td>63.42</td>
<td>9.83</td>
<td>97.42</td>
<td>12.17</td>
</tr>
<tr>
<td>OCs Anx</td>
<td>101.44</td>
<td>15.16</td>
<td>112.68</td>
<td>19.96</td>
</tr>
<tr>
<td>OCs Norm</td>
<td>63.54</td>
<td>10.32</td>
<td>100.73</td>
<td>13.87</td>
</tr>
</tbody>
</table>

Note: Anx: anxious, Norm: non-anxious, Non-Ovul: non-ovulatory, OCs: oral contraceptives, SD: standard deviation. * Indicates significant effect of Anxiety. Anxious individuals had higher scores on the POMS (p < 0.001) compared to non-anxious individuals. Anxious individuals had lower scores on the positive scale of the PANAS (p < 0.001) and higher scores on the negative scale of the PANAS (p < 0.001), compared to non-anxious individuals.

Table 6. Correlations Between Mood Assessments and Behavioral Tasks

<table>
<thead>
<tr>
<th>Behavioral Task</th>
<th>POMS Correlation (p-value)</th>
<th>PANAS-Positive Correlation (p-value)</th>
<th>PANAS-Negative Correlation (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VMWT Distance Block 1</td>
<td>0.04 (0.54)</td>
<td>-0.12 (0.13)</td>
<td>0.07 (0.31)</td>
</tr>
<tr>
<td>VMWT Distance Block 2</td>
<td>0.02 (0.77)</td>
<td>-0.12 (0.10)</td>
<td>-0.01 (0.95)</td>
</tr>
<tr>
<td>VMWT Distance Block 3</td>
<td>-0.02 (0.75)</td>
<td>0.00 (0.96)</td>
<td>0.09 (0.24)</td>
</tr>
<tr>
<td>VMWT Percent in Correct Quadrant (Probe)</td>
<td>0.10 (0.17)</td>
<td>-0.01 (0.89)</td>
<td>-0.01 (0.95)</td>
</tr>
<tr>
<td>HVLT Discrimination Index</td>
<td>0.07 (0.30)</td>
<td>-0.03 (0.72)</td>
<td>0.07 (0.35)</td>
</tr>
<tr>
<td>Verbal Fluency</td>
<td>-0.01 (0.92)</td>
<td>0.08 (0.30)</td>
<td>-0.11 (0.11)</td>
</tr>
</tbody>
</table>
3.4 Conclusions

The results of the study demonstrated a sex difference in spatial navigation behavior that has been seen before. On the VMWT, men had a greater percent of distance in the quadrant that previously held the platform, compared to women, on the probe trial. There were no differences in behavior among women in the ovulatory group, non-ovulatory group, and women on OCs. Participants in this study only completed learning trials followed by a probe trial. In comparison to Study 1, the current study only examined acquisition and not retention of the task. In Study 1, there were no group differences in distance to find the platform or in probe trial performance for the three groups of women. Thus, it is not surprising that there were no group differences in the three groups of women in this study.

No differences were found in the verbal memory tasks. Even though studies have shown sex differences on verbal recollection and recognition (Kramer et al., 1988; Lewin et al., 2001; Munro et al., 2012; Weiss et al., 2003; 2006), there were no group differences in performance on the HVLT. The average overall score for the recollection trials of the HVLT ($M = 26.56, SD = 3.44$) were comparable but slightly lower than the expected normalized data ($M = 29.40, SD = 3.70$) for individuals between the ages of 17 and 30 years old (Benedict et al., 1998). The overall recognition score on the HVLT in this study ($M = 11.53, SD = 0.70$) was in line with the expected score ($M = 11.00, SD = 1.40$; Benedict et al., 1998). Given the similarity in recognition scores, it is surprising that no group differences were seen in this study. No group differences were observed on the verbal fluency task as well. While the conventional letters (F, A, and S) were used in this study (Weiss et al., 2006), some research has shown that sex differences are not always present on verbal memory tasks like verbal fluency (Wallentin, 2009). The average overall score for verbal fluency in this study ($M = 40.66, SD = 8.22$) was also lower than the expected outcome ($M = 43.00, SD = 9.25$;
Weiss et al., 2006). Finally, general mood, assessed by various scales, did not impact behavior on any of tasks for any groups. However, anxiety and mood were related.

Overall, results from Study 2 indicated that general anxiety had no impact on behavioral performance for spatial or verbal memory. Furthermore, performance on any task, or impact of anxiety, did not differ between women in the ovulatory phase, non-ovulatory phase, or on OCs at the time of testing. While men performed better than women on the VWMT, as expected, there were no sex differences on the two verbal tasks. Findings from this study also showed that anxiety was related to mood. Specifically, individuals with high anxiety expressed more negative moods whereas, individuals with low anxiety expressed more positive moods. This suggests that while anxiety did not impact memory, it is associated with general mood.

IV. How Induced Stress Differentially Affects Men & Women on Spatial Memory (Study 3)

4.1 Introduction

Prior literature has shown that sex differences are highly prevalent in spatial memory (Astur et al., 1998; Galea & Kimura, 1993; Moffat et al., 1998; Postma et al., 2004; Sandstrom et al., 1998). In rodents, male rats had impaired spatial memory in response to a chronic stressor, while female rats had enhanced spatial memory in response to stress (Beck & Luine, 2002; Bowman et al., 2001; Conrad et al., 2003; Kitraki et al., 2004; Luine et al., 1994; 1996). In humans, although some research has shown that men and women respond differently to induced acute stress (Andreano & Cahill, 2006; Guenzel, et al., 2014; Mazurka et al., 2017; Zorawski et al., 2006), it remains unclear how spatial memory is affected.

Researchers have also examined the effects of acute stress across the menstrual cycle and have found that women at the phase during which estradiol levels are low are more susceptible to the potential negative effects of stress, whereas those in the high estradiol phases are able to
combat the harmful influences of stress (Albert et al., 2015; Andreano et al., 2008; Antov & Stockhorst, 2018; Bowman, 2005; Espin et al., 2013; Kuhlmann & Wolf, 2005; Maeng & Milad, 2016). For example, by use of the TSST, researchers found that women with low estradiol levels had a significant increase in cortisol, in comparison to women with high estradiol. Increase in cortisol level and overall stress for those with low estradiol was correlated with poorer memory retrieval on an emotional paired association task (Maki et al., 2015). This study mimics what other researchers have shown in that women with low levels of estradiol had increases in cortisol (Andreano et al., 2008; Espin et al., 2013) that was negatively correlated with verbal memory (Espin et al., 2013) and declarative memory (Antov & Stockhorst, 2018).

The goal of this study was to examine how induced stress using a virtual version of the TSST (vTSST) would affect spatial memory in women and men. The VMWT was chosen as a test for spatial navigation given the predominant male advantage (Astur et al., 1998; Astur et al., 2004). To account for sex advantages in spatial memory, an object location task was also assessed since women have been shown to perform better on this task compared to men (Honda & Nihei, 2009; Levy et al., 2005; Spiers et al., 2008). Prior work has shown that women had better memory on a traditional object location task (Honda & Nihei, 2009) and a virtual version of the task (Spiers et al., 2008), compared to men.

Additionally, on a spatial working memory task, Hampson & Morley (2013) found no sex differences between number of errors men and women with high levels of estradiol produced in a delay condition. However, they found that women with low levels of estradiol produced the highest number of errors across all trials compared to women with high levels of estradiol, indicating poorer performance (Hampson & Morley, 2013). Given the results of this study and prior research that has shown that women with low estradiol have a greater stress response that is
negatively correlated with poorer performance (Andreano et al., 2008; Antov & Stockhorst, 2018; Espin et al., 2013), it was expected that women with lower estradiol levels in non-ovulatory phases would not perform as well as women in the ovulatory phase and men by using an adapted version of the spatial working memory task.

As an additional measure of a different type of spatial memory assessment, the mental rotation task was used to assess the impact of stress on sex differences in spatial rotation. This is a non-navigation and non-object location based task that requires mental manipulation of objects in space. Prior literature has shown a male advantage on this task (Astur et al., 2004; Collins & Kimura, 1997; Halpern, 2013; Linn & Petersen, 1985) and that women with lowered levels of estradiol perform better than women with high levels on this task (Hausmann et al., 2000; Mantyla 2013; McCormick & Teillon, 2001).

For the VMWT, a sex difference in favor of men prior to induction of stress, as seen in the literature (Astur et al., 1998; Astur et al., 2004) and in study 2 was expected. Based on prior literature that suggests that men typically have a greater stress response (Andreano & Cahill, 2006; Guenzel, et al., 2014; Mazurka et al., 2017; Zorawski et al., 2006) and research in non-humans that shows impairments in spatial memory due to stress exposure (Beck & Luine, 2002; Conrad et al., 2003; Kitraki et al., 2004; Luine et al., 1994; 1996), it was predicted that men would have a greater stress response to acute stress induction via vTSST and would perform more poorly on the VMWT post-test, compared to control men. In contrast, it was expected that women would either than enhanced memory or be unaffected by the induction of stress and that performance on the VMWT will not differ between the stress and control groups of women. In considering menstrual phases and OC use in women, it was hypothesized that women in the
ovulatory group would be unaffected by stress induction but that women in the non-ovulatory group and women on OCs would have impaired performance due to stress induction.

For object location, it was expected that women would outperform men prior to the stressor (pre-test) and following the stressor (post-test). However, it was expected that induced stress would impair memory post-test for stressed men compared to control men. It was hypothesized that women would be unaffected by stress induction post-test and that they would perform just as well as control women. In considering groups of women, it was hypothesized that women in the ovulatory group would be unaffected by stress induction but that women in the non-ovulatory group and women on OCs would have impaired memory due to stress induction.

For the spatial working memory task, it was predicted that stress induction would impair behavior in men but not in women compared to each respective control group. Furthermore, in considering estradiol levels within groups of women, it was hypothesized that women in the ovulatory group would be unaffected by stress induction but that women in the non-ovulatory group and women on OCs would have impaired memory due to stress induction.

Finally, for the mental rotation task, it was expected that control men would perform better than control women. However, it was predicted that stressed men would perform more poorly than control men on the task, whereas stressed women would perform just as well as control women on this task. Additionally, it was hypothesized that women in the ovulatory group would be unaffected by stress induction but that women in the non-ovulatory group and women on OCs would have impaired mental rotation ability due to stress induction.

4.2 Method

4.2.1 Participants. A total of 336 male and female undergraduates ($M = 18.96$ years, $SD = 1.15$) were recruited from the University of Connecticut. Subjects received class credit for their
participation. Out of the participants that initially consented, 61 participants were excluded for various reasons (cybersickness, irregular menstrual cycles, menstrual cycle phase with likely medium levels of estradiol, use of IUDs, current or previous diagnosis of an anxiety, stress, or depressive disorder – based on self-report, or incomplete data sets). Of the remaining 275 participants, 182 were women. Participants were randomly assigned into a stress or control condition. In considering sex and condition, there were four groups: control women, control men, women who underwent induction of stress, and men who underwent induction of stress.

Female participants were further categorized into hormone groups based on information provided about menstrual cycle and birth control use. The date of last menstruation was subtracted from the study date to calculate the approximate menstrual cycle phase of each participant. With the start of menstruation characterized as day 0, those that were between days 0 to 8 or within days 25 to 35 were considered to be in the non-ovulatory phase. These women would have low estradiol levels given that the day ranges include those in the late-luteal and early follicular phases. Those that were between days 10 to 17 were considered to be in the ovulatory phase. These women would have high estradiol levels which is indicative of the late-follicular and ovulatory phases. Women between any of the remaining days were part of the initial exclusions due to likely have medium levels of estradiol. The cut-offs for each group were based on prior work that show reliable methods of calculating menstrual cycle phases (Mihm et al., 2011). For OC users, women had to have been on a specific type to be eligible for the study. Only monophasic OCs with an ethinylestradiol level that ranged between 0.020-0.035 mg and the same type of progestin (norethindrone) that ranged between 1.00-1.25 mg, were included. None of the women reported being pregnant at the time of testing. The additional groups of women yielded eight total groups for this study (Table 7).
Table 7. Participant Groups for Study 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovulatory</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Non-ovulatory</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Contraceptives</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Excluded</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>101</td>
<td>50</td>
</tr>
<tr>
<td>Ovulatory</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Non-ovulatory</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Contraceptives</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Excluded</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

Note: Women were excluded if there were in a menstrual phase that did not fall within the ranges of the ovulatory or non-ovulatory phases.

4.2.2 Procedure. After receiving informed consent, participants answered a general questionnaire. Following this, participants completed the following assessments: the STAI, the POMS and the Perceived Stress Scale (PSS), which measures perception of stress (Cohen et al., 1983). The STAI is used to determine general anxiety levels, the POMS is used to control for any differences in mood, and the PSS is used as a measure of self-assessed general stress. Physiological measures were then set up. Electrocardiogram (ECG) was obtained via two vinyl electrodes attached on the upper left pectoral and the lower right abdomen, to measure baseline and changes in heart rate. Participants then completed the pre-test of the VMWT, the pre-test of the object location task, either the stress or control task, the post-test of the VMWT, the post-test of the object location task, and then the spatial working memory task. For a subset of participants, the mental rotation task was added following the SWMT, prior to the final subjective survey (Figure 10). The VMWT and object location task were administered prior to the stressor to examine any pre-existing difference in behavior, such as a prevalent sex difference. Both tasks were given again post-test to assess how any previous group differences were impacted by stress. Specifically, the change in behavioral performance was assessed in the
VMWT and the recollection of objects was examined on the object location task. The spatial working memory and mental rotation tasks were given following the stressor only to examine how stress impacts all groups on the task. These tasks were not administered prior to the stressor since practice effects would highly impact the outcome of this task. Subjective measures were collected four times throughout the session in which participants were asked to rate how stressed, anxious, worried, nervous, and bored they were at that moment (Figure 10). Participants rated their subjective feelings on a 5-point Likert scale (1: Not at all to 5: Extremely). The survey was first presented following the demographic questionnaire (initial), then given before the stress or control task (pre-test), then given following the stress or control task (post-test), and finally given one last time at the end of the study session (delayed).

4.2.3 Virtual Morris Water Task. Participants completed the same task that was previously described. However, the pre-test was modified to only have eight hidden platform trials. This modification was done to minimize over-practice with multiple repeated trials. Our prior studies indicated that learning was sufficient with only eight trials. Consequently, the post-test was modified to only have four hidden platform trials for the same reason. Probe trials were given at the end of the pre-test, the beginning of the post-test, and the end of the post-test.
4.2.4 Object Location. Participants were given one minute to initially study an array of objects. They were instructed to pay attention to the location of the objects in relation to one another. Following the study phase, the pre-test was conducted in which participants were given a second array wherein around half of the objects had moved. Participants identified the objects that were in a new location within one minute. Post-test, participants were given the same sheet as given during the pre-test and were allowed one minute to identify any objects that moved compared to the initially studied array. For each trial, the number of incorrect objects was subtracted from the number of correct objects for the total task score.

4.2.5Virtual Trier Social Stress Test. This task ran in a virtual reality platform known as Second Life (borrowed from Fallon et al., 2016). The TSST is a well-established method of inducing stress in a safe but effective manner (Hawn et al., 2015; Jonsson et al., 2010; Kirschbaum et al., 1993). Participants were instructed that they have to give a speech about a hypothetical ideal job to two professors from the university. They were told that this will take place in a virtual environment and that both professors will be listening and interacting within the virtual environment. Participants were initially given two minutes to prepare a speech and allowed to take notes. However, they were told that they will not be able to use notes during the speech. After this, the experimenter removed any notes and left the room so that the participant can give the speech in isolation. Confederates (posing as professors to the participants virtually) were signed into the virtual environment from separate computers. Experimenters controlled the confederates and provided verbal (chat) or non-verbal (gestures) feedback when necessary. Participants were given five minutes to conduct the speech. If they stopped speaking for more than 20 seconds, a confederate encouraged participants to continue speaking using a few vague phrases (ex. “You still have some time. Please continue”, “Say whatever comes to your mind”,
or “Be as creative as you like”). At the completion of five minutes, the confederate let the participant know that time was over. Heart rate was measured during this task.

4.2.6 Control Task. Participants played a simple virtual reality game in which they searched for objects in a virtual reality environment for five minutes to equate to the amount of time in the stress condition. A message appeared that instructed the participant to find a large floating arrow within the room. The participants were to find the arrow and come in contact with it. This occurred multiple times throughout the session.

4.2.7 Spatial Working Memory Task. This task measures both spatial ability and working memory capacity (adapted and modified from Duff & Hampson, 2000; Hampson & Morley, 2013). Spatial ability was measured in that participants had to pay attention to the location of colors in the array on the board. Working memory was assessed in that participants needed to remember which cards had already been selected within a trial. Participants were presented with a 4 by 5 array of blank cards (Figure 11). Hidden behind each card were squares with distinct colors. There was a total of ten matching pairs of colors. Participants revealed one card and then a second, if they matched, they were left uncovered. The goal was to find all of the matching pairs with as minimal errors as possible. Three trials were administered, and the task was timed.

![Figure 11. Depiction of the Spatial Working Memory Task. Participants were to remove two cards at a time. Correctly matching pairs would remain uncovered (top), whereas incorrect pairs would be covered before the next choices. A trial was completed when all matching pairs were revealed (bottom).]
4.2.8 Mental Rotation Task. The same procedure as used in Study 1 was used for this task.

4.2.9 Analyses. To validate that the vTSST induced stress, a correlation between physiological stress (heart rate) and subjective stress (stress questionnaire) was conducted. A positive correlation was expected, which would indicate that as heart rate increased, the subjective stress rating increased as well.

One measure of the VMWT was the distance to find the platform across blocks. For the pre-test, a 2 (Block) by 2 (Sex: men or women) ANOVA was conducted. As another measure, the participants’ percent of distance in the correct quadrant during a probe trial was examined with a one-way ANOVA for Sex. A 2 (Block) by 4 (Group: men, ovulatory women, non-ovulatory women, and women on OCs) ANOVA for distance to find the platform was also conducted. A one-way ANOVA by Group for participants’ percent of distance in the correct quadrant during the probe trial was run. For between group comparisons, Fisher’s LSD post-hoc analyses were conducted.

For the VMWT post-test, a 2 (Condition: stress or control) by 2 (Sex) ANOVA was conducted for distance to find the platform. A 2 (Condition) by 4 (Group) ANOVA for distance to find the platform was also run. For probe trial performance, a difference score was calculated based on the participants’ percent of distance in the correct quadrant at the end of the pre-test versus the percent at the start of the post-test. The difference score assessed changes in performance from the pre-test to the post-test. This difference score was used as the dependent variable for a 2 (Condition) by 2 (Sex) ANOVA and for a 2 (Condition) by 4 (Group) ANOVA. For between group comparisons, Fisher’s LSD post-hoc analyses were conducted.

To analyze performance on the object location task, a difference score was calculated between the pre- and post-test trials of this task. This difference score was the dependent variable for a 2 (Condition) by 2 (Sex) ANOVA and a 2 (Condition) by 4 (Group) ANOVA. For between
group comparisons, Fisher’s LSD post-hoc analyses were conducted. To analyze performance on
the spatial working memory task, a 2 (Condition) by 2 (Sex) ANOVA and a 2 (Condition) by 4
(Group) ANOVA were conducted. Finally, to analyze performance on the mental rotation task, a 2
(Condition) by 2 (Sex) ANOVA and 2 (Condition) by 4 (Group) ANOVA were run.

4.3 Results

4.3.1 Measures of Stress. In analyzing a physiological measure of stress, the average
heart rate (HR) based on beats per minute (BPM) was examined between the stress and control
groups. During the 2-minute baseline, there was no effect of Condition, $F(1, 210) = 2.85$, n.s,
indicating that groups did not differ in heart rate prior to the experiment. However, during the 5-
minute stress or control task, there was an effect of Condition, $F(1, 212) = 27.56 p < 0.001$, such
that participants in the stressed condition had significantly elevated HR during the vTSST
compared to control participants (Figure 12).

![Average Heart Rate Across Time by Condition](image-url)

**Figure 12.** Average heart rate during baseline and during the stress or control task based on
beats per minute (BPM) during each of the 30 second intervals across the 2 minutes of baseline
and 5 minutes of each task. No group differences occurred during baseline. During the task, the
stress group had significantly greater average heart rates during the 5 minutes compared to the
control group ($p < 0.001$).
To examine the change in heart rate, a difference score was calculated between HR during the task and HR during baseline. There was a significant effect of Condition, $F(1, 210) = 64.38, p < 0.001$, indicating that those in the stressed condition had a greater change in HR compared to the control group (Figure 13).

Subjective responses to whether a participant was stressed, nervous, anxious, or worried were combined for an overall subjective stress measure. Results indicated that there was no effect of Condition during the initial, $F(1, 251) = 0.01$, n.s. and pre-test surveys, $F(1, 251) = 0.75$, n.s. However, there was a significant effect of Condition during the post-test, $F(1, 251) = 107.49, p < 0.001$, and the delayed survey, $F(1, 251) = 6.70, p = 0.01$ (Figure 14). These results suggest that participants who underwent the stressor exhibited greater subjective stress compared to control participants immediately following the vTSST and after a delay.

Furthermore, to examine the change in subjective stress, a difference score was calculated between subjective ratings during the post-test survey and the pre-test survey. There was a significant effect of Condition, $F(1, 251) = 127.03, p < 0.001$, indicating that those in the stressed condition had a greater change in subjective stress rating compared to those in the control group (Figure 15).
Additionally, a correlation between physiological stress (heart rate) and subjective stress (stress rating) was conducted. Results indicated that there was a significant positive correlation between HR and subjective rating ($r = 0.39, p < .001$). This indicates that as HR increased, subjective rating of stress also increased.

Furthermore, correlations were run between elevations in HR or subjective stress for each behavioral task. Results indicated no correlations between HR and distance to platform on the VMWT, probe trial performance on the VMWT, object location scores, spatial working memory errors, or mental rotation scores ($p$’s > 0.5).

**Figure 14.** Average subjective stress rating across the experimental session. No group differences occurred for initial or pre-test. The stress group had a significantly higher stress score compared to the control group at post-test ($p < 0.001$) and delayed ($p = 0.01$).

**Figure 15.** Difference score of subjective rating at post-test minus pre-test. The stress group had a significant change in heart rate between sessions ($p < 0.001$), whereas the control group did not.
Results indicated no correlations between subjective stress rating and distance to platform on the VMWT, probe trial performance on the VMWT, object location scores, spatial working memory errors, or mental rotation scores as well ($p$’s > 0.5; Table 8).

Table 8. Correlations Between Stress Measures and Behavioral Tasks

<table>
<thead>
<tr>
<th></th>
<th>Stress Rating</th>
<th>Heart Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VMWT Distance Block 1</strong></td>
<td>Correlation</td>
<td></td>
</tr>
<tr>
<td>(Pre-test)</td>
<td>-0.02</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>($p$-value)</td>
<td>(0.77)</td>
</tr>
<tr>
<td><strong>VMWT Distance Block 2</strong></td>
<td>Correlation</td>
<td></td>
</tr>
<tr>
<td>(Pre-test)</td>
<td>-0.08</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>($p$-value)</td>
<td>(0.21)</td>
</tr>
<tr>
<td><strong>VMWT Distance Block 3</strong></td>
<td>Correlation</td>
<td></td>
</tr>
<tr>
<td>(Post-test)</td>
<td>-0.07</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>($p$-value)</td>
<td>(0.26)</td>
</tr>
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<td><strong>VMWT Percent in Correct</strong></td>
<td>Correlation</td>
<td></td>
</tr>
<tr>
<td>Quadrant – Probe 1 (Pre-test)</td>
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<td>-0.07</td>
</tr>
<tr>
<td></td>
<td>($p$-value)</td>
<td>(0.45)</td>
</tr>
<tr>
<td><strong>VMWT Percent in Correct</strong></td>
<td>Correlation</td>
<td></td>
</tr>
<tr>
<td>Quadrant – Probe 2 (Post-test)</td>
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</tr>
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<td></td>
<td>($p$-value)</td>
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</tr>
<tr>
<td><strong>VMWT Percent in Correct</strong></td>
<td>Correlation</td>
<td></td>
</tr>
<tr>
<td>Quadrant – Probe 3 (Post-test)</td>
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<td>-0.01</td>
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<td></td>
<td>($p$-value)</td>
<td>(0.42)</td>
</tr>
<tr>
<td><strong>Object Location</strong></td>
<td>Correlation</td>
<td></td>
</tr>
<tr>
<td>(Pre-test)</td>
<td>0.08</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>($p$-value)</td>
<td>(0.22)</td>
</tr>
<tr>
<td><strong>Object Location</strong></td>
<td>Correlation</td>
<td></td>
</tr>
<tr>
<td>(Post-test)</td>
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<td>0.00</td>
</tr>
<tr>
<td></td>
<td>($p$-value)</td>
<td>(0.44)</td>
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<tr>
<td><strong>Object Location</strong></td>
<td>Correlation</td>
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<tr>
<td>Difference Score</td>
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<td>0.01</td>
</tr>
<tr>
<td></td>
<td>($p$-value)</td>
<td>(0.67)</td>
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<tr>
<td><strong>Spatial Working Memory Task</strong></td>
<td>Correlation</td>
<td>-0.05</td>
</tr>
<tr>
<td></td>
<td>($p$-value)</td>
<td>(0.42)</td>
</tr>
<tr>
<td><strong>Mental Rotation Task</strong></td>
<td>Correlation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.10</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>($p$-value)</td>
<td>(0.41)</td>
</tr>
</tbody>
</table>
4.3.2 Virtual Morris Water Task. For the pre-test of the VMWT, there was a significant effect of Block, $F(1, 240) = 40.55, p < 0.001$ (Figure 16), such that participant’s distance to find the platform was reduced across blocks. Furthermore, there was a significant effect of Sex, $F(1, 240) = 7.95, p < 0.001$, such that men had shorter distances to find the platform compared to women (Figure 16). However, there was no Block by Sex interaction, $F(1, 240) = 0.69$, n.s.

![Distance to Platform on the VMWT by Sex](image)

**Figure 16.** Mean distance to platform on the virtual Morris water task across the experimental session. Shorter distances indicate more direct paths (better performance). There was an effect of sex in that men had shorter distances compared to women on Block 1 and Block 2 ($p < 0.001$). There were no group differences on Block 3.

The same analyses were repeated for Group instead of Sex. For the pre-test, there was a significant effect of Block, $F(1, 238) = 32.81, p < 0.001$, such that participant’s distance to find the platform was reduced across blocks (Figure 17). Furthermore, there was a significant effect of Group, $F(3, 238) = 2.88, p < 0.001$, such that men had shorter distances to find the platform compared to women in the non-ovulatory group ($p = 0.04$) and women on OCs ($p = 0.006$). There was no difference between men and women in the ovulatory group. Additionally, there was no Block by Group interaction, $F(3, 238) = 0.87$, n.s. (Figure 17).
Figure 17. Mean distance to platform on the virtual Morris water task across the experimental session. Shorter distances indicate more direct paths (better performance). On Block 1 and Block 2 there was an effect of group in that men had shorter distances compared to non-ovulatory women ($p = 0.04$) and OC women ($p = 0.006$).

There was an effect of Sex for the probe trial during the pre-test of the VMWT, $F(1, 238) = 10.11$, $p = 0.002$, such that men had a significantly greater percent of distance in the quadrant that previously held the platform compared to women (Figure 18). Furthermore, there was an effect of Group for the probe trial, $F(3, 238) = 4.28$, $p = 0.006$. Post-hoc analyses revealed that men had a significantly greater percent of distance in the correct quadrant compared to the non-ovulatory group ($p = 0.01$) and the OC group ($p = 0.001$; Figure 19). Men and women in the ovulatory group did not differ in the percent of distance in the correct quadrant.

For the post-test of the VMWT, there was no significant effect of Sex for distance to platform, $F(1, 237) = 2.29$, n.s (Figure 16). The first probe trial at the start of the post-test task measures what was remembered from the pre-test task. There was a significant effect of Sex, $F(1, 233) = 13.02$, $p < 0.001$, and Condition, $F(1, 233) = 7.84$, $p < 0.001$, for this probe trial (Figure 18), indicating that for both sexes, the stress groups had a greater percentage of distance in the correct quadrant compared to the control groups. Furthermore, both groups of men had greater percentages than the groups of women. However, there was no Sex by Condition
There was a significant effect of Sex, $F(1, 235) = 5.92$, $p = 0.016$, for the probe trial at the end of the post-test (Figure 18), indicating that Condition no longer affected performance, however men still had a greater percent of distance than women. Additionally, there was no effect of Condition, $F(1, 235) = 0.16$, n.s., nor a Sex by Condition interaction, $F(1, 235) = 0.02$, n.s.

Additionally, to examine the change in performance due to the impact of stress, a difference score was calculated by subtracting the percent of distance in the correct quadrant for the first probe minus the probe at the start of the post-test task. There was a significant of Condition, $F(1, 232) = 9.85$, $p = 0.002$ (Figure 20), such that the stress groups had lower difference scores indicating that the control groups had a large decrease in percent of distance in the correct quadrant from the first probe (pre-test) to the second (post-test). There was no effect of Sex, $F(1, 233) = 0.38$, n.s., nor an interaction between Condition and Sex, $F(1, 233) = 2.42$, n.s.
n.s. This indicates that the induced stress aided in retention of the platform’s location for those individuals compared to the participants in the control groups.

The same analyses were repeated for Group instead of Sex. There was no effect of Group for distance to platform for the post-test block of the VMWT, $F(3, 233) = 0.80$, n.s. (Figure 17). There was a significant effect of Group, $F(3, 229) = 4.46, p = 0.005$, and Condition, $F(1, 229) = 9.03, p = 0.003$, for the probe trial at the

**Figure 19.** Percent of total distance in the quadrant that contained the platform during hidden trials for all probe trials on the virtual Morris water task. Probe 1: Men had greater percentages in the correct quadrant compared to the non-ovulatory group ($p = 0.01$) and the OC group ($p = 0.001$). Probe 2: Stressed groups performed better than the control groups ($p = 0.003$). Men had greater percentages in the correct quadrant compared to the ovulatory group ($p = 0.05$), the non-ovulatory group ($p = 0.004$), and the OC group ($p = 0.001$). Probe 3: No effect of Group or Condition.
start of the post-test task, such that the stressed groups had better probe trial performance than those in the control condition (Figure 19). Furthermore, post-hoc analyses revealed that men performed better than all groups of women. Men had significantly greater percentages in the correct quadrant compared to the ovulatory group ($p = 0.05$), the non-ovulatory group ($p = 0.004$), and the OC group ($p = 0.001$). However, there was no Group by Condition interaction, $F(3, 229) = 1.03$, n.s. There was no effect of Group, $F(3, 231) = 1.98$, n.s., Condition, $F(1, 231) = 0.14$, n.s., nor an interaction, $F(3, 231) = 0.03$, n.s. for the final probe at the end of the post-test task (Figure 19).

Additionally, to examine the change in performance due to the impact of stress for Group, a difference score was calculated by subtracting the percent of distance in the correct quadrant for the first probe minus the probe at the start of the post-test task. There was a significant of Condition, $F(1, 228) = 8.09$, $p = 0.005$ (Figure 21), such that the stress groups had lower difference scores indicating that the control

![VWMT Difference Score (Probe 2 - Probe 1) by Sex](image)

**Figure 20.** Difference score between the percent of distance in the correct quadrant during the probe trial at the end of the pre-test (probe 1) minus the percent of distance in the probe trial at the start of the post-test (probe 2). There was an effect of Condition in that both control groups had a greater difference score, indicating poorer performance during probe 2, compared to both stress groups ($p = 0.002$).
groups had a large decrease in percent of distance in the correct quadrant from the first probe (pre-test) to the second (post-test). There was no effect of Group, $F(3, 228) = 0.52$, n.s., nor an interaction between Condition and Group, $F(3, 228) = 1.82$, n.s. This indicates that the induced stress aided in retention of the platform’s location for those individuals compared to the participants in the control groups.

![VWMT Difference Score (Probe 2 - Probe 1) by Group]

**Figure 21.** Difference score between the percent of distance in the correct quadrant during the probe trial at the end of the pre-test (probe 1) minus the percent of distance in the probe trial at the start of the post-test (probe 2). There was an effect of Condition in that the control groups had a greater difference score, indicating poorer performance during probe 2, compared to the stress groups ($p = 0.005$).

### 4.3.3 Object Location

For the pre-test trial of this task, a one-way ANOVA for Sex was not significant, $F(1, 249) = 0.01$, n.s., indicating that all groups did just as well on the first trial of object location (Figure 22). For the post-test trial of this task, there was a significant effect of Sex, $F(1, 246) = 5.86$, $p = 0.016$, such that women had significantly greater scores than men.
There was no effect of Condition, $F(1, 246) = 3.49$, n.s., nor an interaction between Condition and Sex, $F(1, 246) = 3.02$, n.s. (Figure 22). In calculating a difference score to measure how much participants were able to retain after the stress/control task, participants’ scores from the post-test trial were subtracted from the pre-test trial. Results indicated that there was a significant effect of Sex, $F(1, 247) = 11.47$, $p = 0.001$, a significant effect of Condition, $F(1, 247) = 4.79$, $p = 0.03$, and a significant interaction, $F(1, 247) = 6.70$, $p = 0.01$ (Figure 23). The finding shows that women had smaller difference scores compared to men,

**Figure 22.** Average object location score across the experimental session. For pre-test there were no group differences. For post-test, women had significantly greater scores than men ($p = 0.016$). There was no effect of condition (stress or control) for the post-test trial.

**Figure 23.** Objection location differences scores from the post-test minus the pre-test. Women had smaller difference scores (better performance) than men ($p = 0.001$). For men, the control group had a significantly smaller difference score than the stress group ($p = 0.01$).
indicating better memory. Furthermore, control men performed better than stressed men. Overall, this suggests that stress impaired performance for men on the post-test trial.

The same analyses were repeated for Group instead of Sex. There was no effect of Group on a one-way ANOVA for the pre-test trial, $F(3, 247) = 0.26$, n.s., indicating that all groups did just as well on the first trial of object location (Figure 24). For the post-test trial of this task, there was no effect of Group, $F(3, 242) = 2.06$, n.s., no effect of Condition, $F(1, 242) = 1.88$, n.s., nor

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**Figure 24.** Average object location score across the experimental session. There were no differences between any groups for the pre-test or post-test trials.
an interaction between Condition and Group, \( F(2, 242) = 1.75, \text{n.s} \) (Figure 24). For the difference score, results indicated a significant effect of Group, \( F(3, 243) = 4.23, p = 0.001 \) (Figure 25). Post-hoc analyses revealed that men had significantly larger difference scores, indicating impaired memory, compared to ovulatory women \( (p = 0.022) \) and women on OCs \( (p = 0.003) \).

There was no effect of Condition, \( F(1, 242) = 1.18, \text{n.s.} \), nor an interaction, \( F(3, 243) = 2.35, \text{n.s.} \).

4.3.4 Spatial Working Memory Task. In a repeated measures ANOVA for 3 trials there was a significant effect of Sex, \( F(1, 226) = 10.18, p = 0.002 \), such that women had significantly fewer working memory errors compared to men (Figure 26). There was no effect of Condition, \( F(1, 226) = 0.09, \text{n.s.} \), nor an interaction, \( F(1, 226) = 0.04, \text{n.s.} \). For the same analysis, there was a significant effect of Group, \( F(3, 222) = 4.21, p = 0.006 \) (Figure 27). Post-hoc analyses revealed

![Diagram](image-url)
that men had significantly more working memory errors than the ovulatory group \((p = 0.019)\) and the non-ovulatory group \((p = 0.001)\).

\[ \text{Figure 26.} \quad \text{Average number of working memory errors in the spatial working memory task across trials. Overall, women had significantly fewer errors (better performance) compared to men \((p = 0.002)\). There was no effect of condition (stress or control).} \]

\[ \text{4.3.5 Mental Rotation Task.} \quad \text{This was task was added later in the study and yielded a sample of 72 participants (36 women). Furthermore, not enough data was collected to form the additional groups of women. There was a significant effect of Sex, } F(1, 68) = 5.40, p = 0.023, \text{ in that men had significantly greater scores compared to women (Figure 28). There was no effect of Condition, } F(1, 68) = 0.04, \text{n.s.}, \text{ nor an interaction between Condition and Sex, } F(1, 68) = 0.01, \text{n.s.}, \text{ indicating that induction of stress did not impact mental rotation ability.} \]
Figure 27. Average number of working memory errors in the spatial working memory task across trials. Men had significantly more working memory errors compared to the ovulatory group ($p = 0.019$) and the non-ovulatory group ($p = 0.001$).
4.4 Conclusions

The findings of this study showed sex differences in almost all tasks and an impact of acute stress exposure for specific measures. Men performed better than women on distance to find the platform and probe trial performance on the VMWT. For both sexes, induction of stress protected probe trial performance from decay at the start of the post-test session, compared to the respective control groups. On the object location task, women performed better than men during the post-test session. Additionally, there was a Sex by Condition interaction in which men had greater difference scores, indicating impaired memory, compared to women. Stressed men also had impaired memory compared to control men. Neither the SWMT nor MRT showed an effect of stress induction. However, performance on the SWMT favored women whereas performance on the MRT favored men.

This study found that the vTSST was a valid measure of stress induction that led to elevations in physiological stress (heart rate) and increased rating of subjective stress. For both measures of stress, participants who underwent the vTSST exhibited significantly greater stress...
scores than the control group. Additionally, both measures of stress were positively correlated with each other ($r = 0.39, p < .001$). This suggests that as heart rate increased, subjective stress increased as well and vice versa. However, neither measure correlated with behavioral performance on any of the tasks. The findings for the change in HR in response to the vTSST further validate the implications of the initial use of the stressor in which researchers found that it elicited increases in cortisol and subjective measures (Fallon et al., 2016). Furthermore, the subjective survey used in Study 3 was novel and was able to yield group differences and correlated with HR, suggesting that the subjective survey was a reliable measure of stress.

Results from the pre-test VMWT were as expected. There was a sex difference in favor of men in that they not only had shorter distances to find the platform, but they also had a greater percentage of distance in the correct quadrant during the probe trial. This sex difference on the VMWT was evident in prior research (Astur et al., 2004). In analyzing for Group instead of Sex, there was a main effect of Group, such that results indicated that men only performed better than the non-ovulatory group and the OC group for both distance to platform and probe trial performance. The ovulatory group did not significantly differ from the men indicating that this group perhaps performed differently than the other two groups of women. Other studies have also found that women with high levels of estradiol have outperformed women with low levels of estradiol on a variety of spatial memory tasks (Bartholomeusz et al., 2008; Hampson & Morley, 2013; Solis-Ortiz & Corsi-Cabrera, 2008).

For the post-test VMWT, there was no effect of Sex or Group for distance to find the platform. This indicates that any sex differences on this task may only be prevalent during acquisition of the task and not during retention. However, in examining the first probe at the start of post-test, a measure of what information is remembered, there is an effect of Sex and
Condition. While men still had greater percentages in the correct quadrant than women, both stressed groups had significantly greater percentages compared to their control counterparts. This suggests that probe trial performance was enhanced for both men and women following exposure to an acute stressor. While this supports the hypothesis for women in which it was predicted that women would have enhanced memory as a result of induced stress, it was an unexpected finding for men. However, prior studies have shown that men had improved memory following and acute stressor on a variety of tasks (Andreano & Cahill, 2006; Zorawski et al., 2006). For the final probe trial at the end of the post-test, the effect of condition disappeared. However, the sex difference in favor of men remained. This indicates that perhaps the induction of stress did not impact probe trial performance after additional learning trials during the post-test. Results for Group replicated what was found for Sex. All three groups of women performed similarly and were impaired compared to men. Based on these observations, it seems as though any behavioral benefit from acute stress induction only impacted the probe trial at the start of the post-test.

Additionally, in calculating a difference score between performance on the probe trial at the start of the post-test (Probe 2) and the probe trial at the end of the pre-test (Probe 1), results indicated an effect of Condition. Stressed groups (for both sexes) did not have as large of a reduction in percent of distance in the correct quadrant as the control groups, demonstrating better memory. Both control groups had larger decreases in the percent of distance spent in the correct quadrant from Probe 1 to Probe 2. Once again, this shows that induction of an acute stressor helped men and women retain the memory for the platform location from the end of the pre-test to the start of the post-test.

The findings from the object location task indicated no group differences during the pre-test trial. This indicates that all groups were able to successfully identify the locations of objects
immediately following the study phase. However, results from the post-test trial indicated that women performed significantly better than men regardless of Condition. These results replicated prior findings that women typically outperform men on this task (Honda & Nihei, 2009; Levy et al., 2005; Spiers et al., 2008). In analyzing Group instead of Sex, there was no effect of Group on object location performance. In examining the change in performance across trials for the object location task, a difference score was calculated. There was an effect of Sex, an effect of Condition, and an interaction between Sex and Condition. The results suggested that stressed women were unaffected by the induction of stress and performed just as well as the control women. However, stressed men performed significantly worse than control men. In this case, stressed men had impairments object location memory in response to an acute stressor. This supports the hypothesis in that men typically experience the negative effects of stress more profoundly than women. Interestingly, this was only the case for object location memory. This was not evident from spatial navigation behavior on the VMWT. In examining the difference score for Group, men had larger difference scores and impaired memory compared to the ovulatory women and women on OCs. Men did not differ from non-ovulatory women for difference score on this task. These findings suggest that women in the ovulatory phase, in which estradiol levels are elevated, and women on OCs, that have a steady level of estradiol, had enhanced performance on the object location task compared to men, regardless of condition (stress or control).

For the spatial working memory task, results yielded a significant effect of Sex on the number of working memory errors across trials. Specifically, women had significantly fewer errors and better memory than men on this task. Although this outcome is different than what Hampson & Morley (2013) found in their study in using this task, it is important to note that the
task was modified in Study 3. While working memory errors were calculated in the same method, the task had slight differences. For example, in the original task, correct pairs of colors were covered prior to proceeding to the next possible pair (Hampson & Morley, 2013). In the current study, correctly identified pairs remained uncovered until all pairs were found. This difference could have impacted the results of the study. Thus, the sex difference that occurred in working memory errors indicates that the current version of this task has an advantage for women instead of men. In considering Group instead of Sex, results indicated that only the ovulatory and non-ovulatory groups (the two naturally cycling groups) had significantly fewer errors than men. The OC group did not significantly differ from men in the number of errors. This suggests that women with naturally cycling endogenous estradiol had enhanced performance on this task compared to men. Surprisingly, there was no effect of Condition on the SWMT. Induced stress did not impair or enhance behavior for any group. This suggests that perhaps spatial working memory is less sensitive to the impact of an acute stressor.

For the final behavioral task, a sex difference on the mental rotation task was observed. As expected, men had significantly greater scores on the MRT compared to women. These findings support what has been found in previous research (Astur et al., 2004; Collins & Kimura, 1997; Halpern, 2013; Linn & Petersen, 1985). Since the MRT was only given to a subset of participants, not enough data was collected to examine the effects of Group. There was no effect of Condition on MRT score. These results suggest that perhaps mental rotation ability is not vulnerable to changes by acute stress.

Overall, the findings from this study indicate that induction of an acute stressor via the virtual version of the TSST, led to elevations in HR and increased subjective stress. The impact of stress was seen on the VWMT, which tests for spatial navigation, and on the object location
task, which tests for spatial locations of objects. Induced stress protected probe trial performance at the start of the post-test VMWT for both men and women. However, for the object location task, women were unaffected by the stressor, whereas men were hindered by stress exposure. The vTSST did not impact performance on the SWMT, which tests for working memory of spatial locations, nor the MRT, which tests for mental rotation ability. While sex differences were apparent in spatial navigation (VMWT) and mental rotation in favor of men, women performed better on object location and spatial working memory.

V. General Discussion

The collection of studies discussed provides vital information on how a variety of factors, including estradiol levels, anxiety, and stress impact behavioral performance on hippocampal-dependent spatial memory tasks in young adult men and women. To examine the outcomes of how these factors influence spatial memory, three studies were conducted with individual objectives. Study 1 assessed how levels of estradiol in women impacted spatial memory performance. Retention after 24 hours was also tested. Study 2 examined how general anxiety levels impacted spatial and verbal memory. Sex differences were also analyzed in addition to menstrual cycle phase and OC use in women. Finally, Study 3 investigated how the induction of an acute stressor affected spatial memory in men and women while examining sex differences, menstrual cycle phase in women, and OC use in women.

5.1 Effects of Estradiol on Behavioral Performance

5.1.1 Categorization of Estradiol. All three studies assessed the impact of estradiol on learning and memory. For Study 1, estradiol levels were obtained from each participant via saliva assay to determine the exact value at the time of testing. In Studies 2 and 3 saliva assays were not obtained due to financial restrictions. Instead, menstrual cycle information was used to
calculate the approximate menstrual phase in which each participant was in during time of testing. Although, a standard method of calculating menstrual cycle phase was used for these studies (Mihm et al., 2011), it is possible that there were errors in determining groups of women. It was assumed that participants provided accurate and valid information about their menstrual cycle. Additionally, because the information was obtained in self-reported measures, it is possible that some participants made errors in estimating the date of their last menstrual. However, both Studies 2 and 3 asked participants for their confidence level in estimating their last menstruation to try and minimize report error. Out of a possible 10, the average confidence level for Study 2 was 8.91 (SD: 1.57) and for Study 3 was 8.78 (SD: 1.52). Confidence was high for both studies, likely indicating that participants provided accurate information. Furthermore, once women were categorized into the ovulatory and non-ovulatory groups, it was assumed that these women had typical cycles with the typical average levels of estradiol and progesterone across their cycles. Women in any other phase were not used for analysis to better predict high levels of estradiol in the ovulatory phase and low levels in the non-ovulatory phase. Both groups were also assumed to have low levels of progesterone. Furthermore, given the high prevalence of OC use in women (Chadwick et al., 2012; Daniels et al., 2014), participants using specific types of monophasic OCs, with a defined estradiol level, progesterone level, and progesterone type were also included in all three studies. Participants once again provided the OC type by self-report. It is possible that there were errors in reporting the incorrect type of OC, length of time on OC, or not reporting use of an OC at all. However, given that participants were aware that this information would be requested during the experiment when signing up for the study, most participants were prepared to provide the relevant information.
5.1.2 Spatial Navigation. To examine behavioral differences amongst groups on spatial navigation performance, participants completed the VMWT for all three studies. For Study 1, results for the VMWT showed no group differences in either distance to platform or probe trial performance on day one. This suggests that acquisition of the task is likely not sensitive to the differences in estradiol level. Others have also found that women with high estradiol, low estradiol, or on OCs did not differ in acquisition of fear conditioning (Graham & Milad, 2013). Similarly, one study found that women in the early follicular, ovulatory, and mid/late luteal phases of their cycle performed comparably during learning trials of a virtual radial arm maze (Hussain et al., 2016). In assessing the consolidation of memory post-learning in Study 1, women were re-tested on the VMWT 24 hours later. Women with high levels of estradiol had better performance than women with low levels of estradiol for both distance to platform and probe trial performance at the start of day two. For probe trial performance at the start of day two, the LE group also performed more poorly than the OC group. No group differences were observed during the probe trial at the end of day two. Our data is consistent with a fear-conditioning study in which women with high estradiol, low estradiol, or on OCs exhibited similar day one acquisition, but the high estradiol group displayed the best extinction memory on day two, 24 hours later (Graham & Milad, 2013). Overall, for the VMWT, it was observed that the LE group had impaired performance at the start of day two. These findings indicate that women with low levels of estradiol during the time of learning and during retention may have a disadvantage on a spatial navigation task that tests for the ability to learn a location of an item by use of spatial cues in an environment.

Prior work has also shown that lowered levels of estrogen hormones have a negative impact on other types of memory as well. In one study, researchers examined women that
received a hysterectomy and bilateral oophorectomy for benign disease (Phillips & Sherwin, 1992). As a result of surgery, estrogen hormones rapidly decrease and often incur unwanted side effects. Cognitive impairments can also occur and were examined in this study. Researchers administered memory tests prior to surgery and again two months post-surgery. Some women received estrogen replacement therapy following surgery and others received a placebo. Results indicated that women treated with estrogen had no change in immediate or delayed recall of paired associations. In contrast, placebo treated women had significant declines in performance on this task from baseline to post-surgery (Phillips & Sherwin, 1992). In another study, researchers tested healthy post-menopausal women who were either on HRT or not, on a variety of cognitive measures (Doty et al., 2015). Results indicated that women who were receiving HRT with both estrogen and progestin had the highest scores on the Wechsler Adult Intelligence Scale and the Neuropsychological Instrument Spatial Span Backwards Test, compared to women not on HRT (Doty et al., 2015). Another study investigated sex differences in older adults (Castonguay et al., 2015). Post-menopausal women taking HRT, post-menopausal women not on HRT, and men were administered multiple executive function tasks. Both women on HRT and men performed better on a two-back task, in which a list of letters was read, and participants had to indicate whether each letter was the same or different than the letter presented two letters back, than women not on HRT. Women on HRT also had better visual divided attention on a task that required participants to correctly identify a figure on a screen, compared to women not on HRT and men (Castonguay et al., 2015). The findings from these studies further imply that drastic reductions in estrogen hormones can lead to some kinds of cognitive impairments and that replacement of estrogen and related hormones may be able to alleviate some deficits.
Others have examined the relationship between the reduction in estrogen hormones during menopause, hormonal replacement treatment, and changes in hippocampal structure (Erickson et al., 2010). Researchers measured hippocampal volume in post-menopausal women. They also assessed whether spatial memory was associated with the time between the onset of menopause and the initiation of hormone treatment. Results indicated that shorter intervals between the onset of menopause and the onset of hormone treatment was associated with larger hippocampal volumes than compared to longer intervals. Women who started treatment early also had larger hippocampal volumes compared to women who never had hormone treatment. However, larger hippocampal volume was not associated with improved spatial memory performance. In fact, hippocampal volume was unrelated to behavior at all (Erickson et al., 2010). While the findings of this study suggest that estrogen hormones are related to structural changes in the hippocampus that occur post-menopause, the relationship that it has with memory is unclear. Future studies should attempt to examine structural changes in the hippocampus as a result of fluctuating estrogen levels across the lifespan and assess how learning and memory are impacted by those changes.

Other work has examined how estrogen replacement therapy affects the diagnosis of AD. Post-menopausal women were assessed across a one-year period and measured for cognitive impairment on a dementia rating scale (Costa et al., 1999). Researchers found that women who were on estrogen replacement therapy had significantly lower rates of AD diagnoses at the end of the one-year period, compared to baseline, than women not on estrogen treatment. Additionally, women on estrogen treatment had no change in cognitive functioning, based on the dementia rating scale, between baseline and at the one-year follow-up, whereas women not on treatment had significant cognitive deficits from baseline to follow-up (Costa et al., 1999). The
outcome of this study implies that replacing estrogen following the rapid reduction that occurs in menopause may be beneficial for possibly preventing or slowing the possible progression of developing Alzheimer’s disease.

Overall, results from Study 1 on the VMWT indicate that estradiol may not be associated with acquisition of this task. Specifically, based on the findings, it appears that estradiol did not impact distance to find the platform across trials on day one nor did it affect the percent of distance in the correct quadrant in the probe trial. This suggests that estradiol was not vital in learning this type of spatial navigation behavior amongst the groups of women. However, the results from day two suggest that estradiol may facilitate memory retention of the platform location. Given that women with high levels of estradiol performed better than women with low levels of estradiol on both distance to platform and probe trial performance, it seems evident that there is a distinction between these two groups in memory consolidation. Particularly, the findings suggest that having low circulating levels of estradiol leads to impairments in retention on this spatial navigation task.

Rodent data also support the notion that high estradiol levels enhance memory consolidation. For example, gonadally intact mice and rats in proestrus (high estradiol levels) exhibit better spatial memory than rats in estrus or diestrus (low estradiol levels) in the Morris water maze (Frick & Berger-Sweeney, 2001) and on an object location task (Frye et al., 2007; Paris & Frye, 2008). In addition, systemic exogenous estradiol treatment in ovariectomized rodents enhances memory consolidation in a variety of memory tasks including the Morris water maze, object placement, and object recognition (Gresack & Frick, 2006; Inagaki et al., 2010; Packard & Teather, 1997). In particular, ovariectomized rats and mice treated with a single systemic injection of 0.2 mg/kg 17β-estradiol immediately after Morris water maze training trials
exhibited enhanced spatial memory relative to controls or rodents treated with 0.1 or 0.4 mg/kg estradiol (Gresack & Frick, 2006; Packard & Teather, 1997). However, the 0.2 and 0.4 mg/kg doses both enhanced consolidation of object recognition memories in ovariectomized mice (Gresack & Frick, 2006; Fernandez et al., 2008), suggesting that different levels of estradiol may be optimal for different tasks or types of memory. These studies illustrate the importance of establishing an optimal dose of estradiol for memory enhancement. For spatial memory as tested in the VMWT, it would appear that high estradiol during the menstrual cycle is optimal.

Unexpectedly, women on contraceptives did not have impaired performance on the VWMT compared to women with high estradiol, as predicted. In fact, the OC group did not differ from either naturally cycling group in distance to find the platform and had better probe trial performance than the low estradiol group at the start of day two. This suggests that OC use does not impact acquisition or retention of spatial memory on this task. Furthermore, the findings indicate that OC use did not lead to any impairments in spatial navigation. Overall, results from Study 1 demonstrate that levels of circulating estradiol in naturally cycling women can impact their spatial memory performance. The OC group performed similarly to the HE group in Study 1, which is in contrast to some prior research. For example, one study found that women on OCs poorer fear memory than naturally cycling women overall and naturally cycling women with low estradiol levels had significantly better memory than OC women (Graham & Milad, 2013). Moreover, the same study reported that the deficit induced by OCs was reversed by terminating OC use or administering estrogen receptor agonists (Graham & Milad, 2013). In contrast, the OC group in the present study was not impaired relative to the low estradiol group, even when a similar testing design was used. Based on prior research, it was expected that the OC group would show some deficit, given that most OCs typically reduce the overall level of estradiol and
progesterone even with the consumption of synthetic forms these hormones (Griksiene & Ruksenas, 2011; Mordecai et al., 2008). However, our results suggest that high levels of estradiol may be more beneficial for women in a natural cycle than low levels. This conclusion is supported by other results indicating that naturally cycling women experienced no change in verbal memory on the California Verbal Learning Test across the menstrual cycle but that OC users exhibited enhanced memory (Mordecai et al., 2008). Other studies have shown that naturally cycling women with high estradiol levels and OC users perform the best on verbal memory tasks (Rosenberg & Park, 2002) and on immediate and delayed memory for verbal lists of words (Gogos, 2013), compared to naturally cycling women with low estradiol levels. Additionally, some studies have shown no differences between OC users and naturally cycling women in working memory (Vranic & Hromatko, 2008).

One distinction between the naturally cycling groups and the OC groups other than level of estradiol is the type of estrogen hormone. Although OC groups typically have a reduced level of estradiol (Griksiene & Ruksenas, 2011; Mordecai et al., 2008), a steady dose of synthetic estradiol in the form of ethinylestradiol is taken exogenously into the system, whereas in naturally cycling women, estradiol is synthesized and released endogenously, which may result in some differences in how estradiol is processed (Beltz et al., 2015; Gogos et al., 2014; Griksiene & Ruksenas, 2011; Mordecai et al., 2008). Furthermore, in ovariectomized mice, estradiol synthesized in and released by the hippocampus is necessary for spatial and object recognition memory consolidation (Tuscher et al., 2016), indicating a potential impact of local synthesis on learning and memory. Thus, it is possible that estradiol synthesized within the hippocampus might differ in functionality from ethinylestradiol derived from OCs. Although the OC group in the current study did not display any spatial memory impairments, these factors
suggest that these differences are not critical in our current spatial navigation tasks.

Furthermore, while strategy use was not examined in Study 1, prior work has shown differences in strategy on navigation tasks across the estrous cycle in rodents and menstrual cycle in women. In one study, juvenile female rats were trained on a T-maze, submerged in water, to find a goal location (Pleil & Williams, 2010). Rats were trained using a place and a response strategy. A place strategy requires the use of spatial cues in the environment to remember the location of the goal. A response strategy requires remembering the direction of movement regardless of spatial cues. Rats were then tested at adulthood at various points of the estrous cycle. Results indicated that female rats that were in the proestrus phase of their cycle used the place strategy to find the goal location rather than the response strategy. Rats in all other phases of their cycle had no differences in strategy use. These findings suggest that when estradiol levels are high (during proestrus), female rats preferred a spatial technique to solve the task rather than a non-spatial method (Pleil & Williams, 2010). Another study found that women in the luteal phase of their menstrual cycle were more likely to use a landmark strategy on a 2-dimensional matrix navigation task than women in the follicular phase of their cycle (Scheuringer & Pletzer, 2017). Additionally, use of landmark strategy led to better performance on the task for women in the luteal phase, compared to women in the follicular phase (Scheuringer & Pletzer, 2017). Results from Study 1 indicated that women with high levels of estradiol had better retention than women with low levels of estradiol on a spatial navigation task. It is possible that the two groups of women used different strategies to solve the task. Future research should consider assessing strategy use to determine how estradiol levels impact spatial navigation.
In Study 2, performance on the VMWT was compared between women in the ovulatory group, women in the non-ovulatory group, women on OCs, and men. The findings indicated an expected sex difference on the VMWT. It was observed that men performed better than women during the probe trial in that men had a greater percent of distance in the quadrant that previously held the platform, compared to women. In comparing groups of women, there was no difference in behavior for distance to platform or probe trial performance, similar to what was seen in Study 1. Participants in Study 2 only completed the VMWT task that participants in Study 1 completed on day one. Results for that study showed no group differences in learning on day one as well. The same acquisition session was also given in Study 3 (although slightly shorter), and once again the three groups of women did not differ from each other. The three groups of women also did not differ in performance on any of the probe trials or hidden platform trials during the post-test session. In comparing these results to Study 1, it is not surprising that the ovulatory group, which has higher levels of estradiol, did not perform better than the non-ovulatory group, which has lower levels, at the start of the post-test session. Even though the HE group performed better than the LE group at the start of day two, there is a large difference in the amount of time passed between the two sessions. In Study 1, retention of memory was tested after 24 hours. In Study 3, memory retention was tested around 10 to 12 minutes after acquisition trials were completed. This short amount of time is likely not enough time for long term memory consolidation. This suggests that any effect of estradiol on memory consolidation occurs at least 12 minutes post-learning and before 24 hours post-learning.

Prior work has also shown no difference in estradiol level on some spatial navigation tasks. In one study, OVX mice were treated with either low estradiol replacement, high estradiol replacement, or placebo (Rissanen et al., 1999). The low dose was chosen to mimic levels
typically observed during estrus whereas, the high dose was chosen to mimic levels typically observed during proestrus. Results indicated that both the low and high estradiol treated groups performed better on a spatial navigation water maze than the placebo treated group. However, there was no difference in performance between the two estradiol treated groups (Rissanen et al., 1999). The findings from this study indicate that estradiol treatment was better than placebo for spatial memory but that amount of estradiol did not affect memory. In this case, having estradiol administration following an ovariectomy at all was beneficial. Many other researchers have previously shown that HRT in general is beneficial for cognition in post-menopausal women as well. Women who received some form of hormonal treatment had fewer working memory errors on a spatial working memory task and on a verbal task and had faster completion times on the spatial working memory task, compared to a control group without hormonal treatment (Duff & Hampson, 2000). HRT was also shown to enhance verbal memory on the California Verbal Learning Test in post-menopausal women taking HRT either with estrogen alone or in conjunction with progesterone (Maki et al., 2001; Resnick & Maki, 2001). Estrogen placement also improved verbal recall in post-menopausal women compared to control women not receiving any hormonal treatment (Robinson et al., 1994).

Results from Study 3 on the VMWT also indicated a sex difference on the task. Men had shorter distances to find the platform during the learning trials (pre-test) than women. Men also had significantly greater distances in the correct quadrant compared to women on all three probe trials. These results replicated what has been seen in prior work (Astur et al., 1998; Astur et al., 2004). It was an unexpected finding that there were no sex differences for distance to platform during the learning trials of Study 2. Study 3 had eight hidden platform trials during which men had shorter distances, than women, across trials. For Study 2, both men and women have around
the same distance during their first eight trials. What remained consistent was that men had better probe trial performance in both studies, compared to women. This suggests that perhaps the sex difference that occurs on this task is more sensitive to probe trial performance rather than distance to platform. However, distance to platform seems to be reliant on the number of learning trials. Future studies can test whether sex differences in behavioral performance on this task is affected by number of trials during acquisition.

Participants in Study 1 were also tested on the RAM as an additional measure of spatial navigation. The RAM assesses working and reference memory errors in addition to distance to obtain rewards across trials. Results from Study 1 revealed no group differences on this task. The observed lack of estradiol effects in working memory errors is consistent with previous work in gonadally-intact rats (Stackman et al., 1997), but not with prior research in rats in which systemic estradiol treatment improved spatial working memory in a radial arm maze among ovariectomized rats (Fader et al., 1999; Daniel et al., 1997). The null effects on spatial reference memory are consistent with data from ovariectomized rats treated with exogenous estradiol (Fader et al., 1999). Thus, the current data are consistent with some RAM findings from the rodent literature. However, the findings from Study 1 are not consistent across tasks; that is, the lack of estradiol effects in the RAM was inconsistent with the beneficial effects of high estradiol on spatial memory consolidation observed in VMWT. Our lab has previously shown that virtual spatial navigation tasks do not always produce similar performance across tasks. Specifically, it was previously reported that a male advantage in the VMWT has observed, but no sex differences were seen on the RAM among the same participants (Astur et al., 2004). This discrepancy lends support to the notion that these tasks are not interchangeable in terms of spatial memory ability and are differentially sensitive to memory processes such as reference and
working memory. Future research should examine whether parametric manipulations (e.g. number of arms in the maze, number of rewards provided, delays, etc.) of the radial arm maze task might increase sensitivity to estradiol levels.

5.1.3 Mental Rotation. The impact of estradiol level on mental rotation ability was assessed in Study 1. The findings indicated no difference in performance for the high estradiol, low estradiol, and OC group. While some studies have shown that women in the menstrual cycle phases with low estradiol perform better than women menstrual cycle phases with high estradiol (Hausmann et al., 2000; Mantyla 2013; McCormick & Teillon, 2001), others have shown no differences in performance on the MRT among women at various points of their menstrual cycle or women on OCs (Mordecai et al., 2008; Rosenberg & Park, 2002). Additional work has focused on angles of rotation and the impact of behavior across a menstrual cycle (Hampson et al., 2014). Researchers found that when there were large angles of rotation, women with low estradiol had better performance on the MRT than women with high estradiol. However, when there were small angles of rotation, estradiol did not impact performance (Hampson et al., 2014). These findings suggest that the influence of estradiol level of mental rotation ability may depend on the difficulty of object rotations. However, the overall results from Study 1 suggest that neither estradiol level nor OC use facilitates mental rotation ability. Since level of difficulty was not assessed, future work can examine how degree of angle rotation affects mental rotation ability.

Sex differences in mental rotation ability were assessed in Study 3. The results from Study 3 indicated an expected sex difference on MRT performance in that men performed better than women. Men had higher MRT scores on the task, compared to women; a pattern that has been seen in previous studies as well (Astur et al., 2004; Choi & Silverman, 2003; Halpern,
2013; Linn & Petersen, 1985; Moffat et al., 1998; Silverman et al., 2000; Wei et al., 2016). Some have also assessed degree of difficulty on the task in how men and women are impacted. Collins & Kimura (1997) design a mental rotation task similar to the one commonly used (Vandenber & Kuse, 1978). They varied task difficulty and found that men only performed better than women when the task was very difficult (major rotations). No sexes were observed when the task was easy (minor rotations). These researchers claim that women were just as successful in processing 3-dimensional rotations; however, task difficulty is likely what leads to the sex difference on mental rotation tasks (Collins & Kimura, 1997). Given that task difficulty was not assessed in the present studies, it is unclear how our participants would have performed if difficulty level was varied. Other work has compared the traditional mental rotation task to one conducted in a virtual environment (Parsons et al., 2004). These researchers found that men performed better than women on the traditional task. However, no sex differences were observed for the mental rotation task in the virtual environment. They believed that the lack of sex difference on the virtual task may be attributed to the differences in stimulus complexity. The virtual task required a more complex target and matching objects. This yielded more potential rotations and configurations. In this case, it seems apparent that the virtual task was more difficult than the traditional MRT and perhaps was equally as difficult for both sexes (Parsons et al., 2004). Overall, prior work suggests that the traditional MRT (Vandenber & Kuse, 1978) is difficult enough to elicit a sex difference, in favor of men, but not too difficult so that men would show impairments. Some work has shown how MRT performance relates to other factors. For example, the superior performance of men on this task was positively correlated with their superior performance on a wayfinding task; a pattern that was not observed for women (Silverman et al., 2000). Others have shown that young adult men had a positive correlation
between mental rotation performance and anterior hippocampal volume (Wei et al., 2016). It is evident from prior work and from the results of Study 3 that men do perform better on the MRT than women. However, it remains unclear why women have impaired performance on this task.

5.1.4 Object Location. Women in the ovulatory group, women in the non-ovulatory group, women on OCs, and men completed the object location task in Study 3. Results indicated no group differences during the immediate trial of testing. However, during the delayed trial, all groups of women performed better than men. The delayed trial was administered around 15 to 17 minutes post-learning. It seems as though women have an advantage in holding on to information for the locations of objects for a longer period of time, compared to men. There were no differences among the three groups of women during either trial. A difference score was calculated to see the change in performance from pre-test to post-test. Results indicated that men had significantly larger difference scores, indicating poorer performance, than ovulatory women and women on OCs. Men did not differ from the non-ovulatory group for difference score. Additionally, stressed men had significantly larger difference scores compared to control men. Overall, it was clear that stressed men had the greatest reduction in object location score from pre-test to post-test. Control men also had a reduction in score between pre-test to post-test however, all groups of women performed similarly between the two trials.

It is evident that men have impaired object location memory compared to women. What remains unclear is why that sex difference occurs. To assess the role of attention, one study tested men and women on real-life object location memory under different conditions (Barel, 2018). To assess incidental memory (unintentional memory for surrounding information), participants were split into a full attention or divided attention condition. For the full attention condition, participants were asked to wait in a room for several minutes. This stimulus room
contained an array of objects. Following this, participants were taken to another room and shown a picture of the array to identify which objects were in a different location. For the divided attention condition, participants had to complete a distractor task while in the stimulus room that involved a simple verbal math task. Following this, they completed the second part as the other group. Results indicated that object location scores in the divided attention group were significantly lower than the full attention group. Furthermore, there was no sex difference in the divided attention condition. However, women had superior object location scores in the full attention condition, compared to men. To assess intentional memory (directed memory for surrounding information), both attention conditions were also employed. Both procedures remained the same with the exception of being instructed to memorize as many objects as possible in the room and their approximate locations. Results indicated that once again scores on the divided attention condition were significantly lower than on the full attention condition. Furthermore, women had significantly greater object location memory scores in the full attention condition, compared to men. No sex difference was observed for the divided attention condition (Barel, 2018). Overall, the findings of this study suggest that women are better able to remember the location of objects than men, when they are fully attending to the objects. Additionally, this advantage occurred regardless of whether participants were instructed to observe the objects and their locations or not. The results from the intentional learning condition with full attention mimic what was seen in Study 3, in that women had better object location memory than men when instructed to study the locations of objects.

In another study, researchers assessed the impact of estradiol and sex differences in object location memory in rodents (Cost et al., 2012). OVX female rats were treated with estradiol and progesterone to mimic levels typically observed in proestrus. Performance was
compared to OVX rats treated with vehicle and male rats. Results indicated that OVX female rats treated with vehicle and male rats had significantly fewer correctly identified object locations compared to OVX female rats treated with estradiol and progesterone (Cost et al., 2012). The results from this study support the results from Study 3. In both cases, women with circulating levels of estradiol and progesterone had better object location memory than men. OVX female rats did not perform well on the task and could be considered similar to post-menopausal women. As prior work has shown, with estrogen replacement, the deficits in cognitive abilities in post-menopausal women can be reduced (Duff & Hampson, 2000; Maki et al., 2001; Resnick & Maki, 2001; Robinson et al., 1994).

5.1.5 Spatial Working Memory. Study 3 also assessed spatial memory on a spatial working memory task. This task, while requires the processing of spatial information, specifically the location of colored squares, also heavily relies on working memory. Results from Study 3 showed that women had fewer working memory errors on the task than men across trials. Upon further examination, analyses revealed that men had significantly greater working memory errors only compared to the ovulatory and non-ovulatory groups. Men and OC women did not differ. Furthermore, there was no difference between the ovulatory and non-ovulatory groups. This finding was in contrast to other work. Joseph and colleagues (2012) found that women in the late follicular phase (similar to our ovulatory group), had fewer working memory errors on an N-back test with varied levels of memory load, than women in the early follicular phase (similar to our non-ovulatory group). Additionally, this study found that superior performance in the late follicular group was associated with reduced brain activation of the left hemisphere and increased recruitment of the right hemisphere, compared to the early follicular group. Activation was measured in various structures including the hippocampus and prefrontal
cortex (Joseph et al., 2012). Although results from Study 3 did not mimic the findings of this study, it is important to consider task differences. Our task is much more dependent on spatial memory and thus may require more hippocampal functioning. Prior work has shown that the prefrontal cortex is heavily recruited in working memory tasks (Hampson, 2018). Specifically, the dorsolateral prefrontal cortex (DLPFC) has been shown to be important in tasks that require the manipulation of information in short-term storage (Barbey et al., 2013). Damage to the DLPFC has also been associated with impaired performance on verbal working memory and spatial working memory tasks (Barbey et al., 2013). Differences in how the prefrontal cortex and hippocampus are recruited together to process spatial working memory may impact behavior.

Additionally, the findings from Study 3 were different than what Hampson and Morley (2013) found in their participants. In their study, women with high estradiol and men did not differ in the number of working memory errors. However, women with low estradiol levels had the greatest number of errors across all trials compared to both men and women with high estradiol (Hampson & Morley, 2013). The result of Study 3 indicated that both naturally cycling groups, women in the ovulatory and non-ovulatory group had significantly fewer working memory errors compared to men. Furthermore, both naturally cycling groups did not differ from the OC group. It is important to note that the spatial working memory task used in Study 3 was modified and may have tested for different aspects of spatial and working memory than the task used in the Hampson and Morley (2013) study. In their study, once a matching pair of colored dots were found, the correct pair was covered before the next selection. Thus, participants had to hold onto more information than required in the task used in Study 3. Participants in the Hampson and Morley study had to not only remember the location of colored dots to obtain future correct pairs, but also had to remember the correct pairs they already matched. In the task
used in Study 3, participants only had to remember the location of colored squares to make new correct matches. They were essentially allowed to ignore the pairs that were already matched, given that they were left uncovered. It is possible that the working memory load for participants in the Hampson and Morley (2013) study was greater than the working memory load in Study 3 since less information had to be remembered in Study 3.

In assessing working memory load one study examined the change in activation in the prefrontal cortex (Rypma & D’Esposito, 1999). Participants completed a task under fMRI in which a group of letters was presented. Following a delay, one letter appeared, and participants had to indicate whether it was part of the original set or not. This occurred for multiple trials. Results indicated that as memory load increased (greater number of letters presented in the set), activation increased in the dorsal prefrontal cortex and was lateralized to the right hemisphere. However, when the memory load was low, there were no differences in activation between the dorsal prefrontal cortex and the ventral prefrontal cortex or between hemispheres (Rypma & D’Esposito, 1999). Another study found similar results (de Fockert et al., 2001). In this study, participants had to ignore distractor faces while holding onto a sequence of digits either in the same order (low memory load) or in a different order (high memory load) on multiple trials. Results indicated that high memory load was associated with greater activation of the prefrontal cortex, more interference from the distractor faces, and increased activation of the visual cortex, compared to the low memory load. These researchers concluded that visual selective attention is highly related to working memory (de Fockert et al., 2001). The findings of these studies imply that working memory load does affect processing in the prefrontal cortex and could impact the subsequent functioning in behavior.
Other work has examined how estradiol treatment affected working memory load in post-menopausal women (Dumas et al., 2010). In this study, healthy post-menopausal women were either given estradiol treatment or placebo for three months. All participants completed a visual verbal N-back sequential letter test during fMRI at baseline and at follow-up, three months later. Results indicated that women on estradiol treatment had increased activation in the frontal cortex during high working memory load, compared to women treated with placebo. There were no differences in activation with low working memory load. Additionally, there were no differences in behavioral performance between the two groups of women (Dumas et al., 2010). The findings of this study suggest that estradiol treatment was able to affect brain activity in the frontal cortex only when working memory load was high. However, it is important to note that differences in activation were unrelated to behavior. Working memory load was not assessed in Study 3. However, future work should examine how the influence of estradiol or sex differences is affected by manipulation of memory load. Furthermore, estradiol level or menstrual cycle phase may have an impact on frontal cortex activation, even if behavioral differences are not observed.

5.1.6 Verbal Memory. Verbal recollection and recognition were assessed on the HLVT in Study 2. Not only were there no sex differences, there were also no differences in performance among women in the ovulatory group, women in the non-ovulatory group, or women on OCs. This was also true for the verbal fluency task, which was an unexpected finding. Prior research observed that women in the ovulatory group produced fewer words on a verbal fluency task compared to women in the late-luteal phase of their cycle, during which estradiol levels are low (Solis-Ortiz & Corsi-Cabrera, 2008). Based on the results from Study 2, it is clear that performance on the HVLT and verbal fluency were unaffected by differences in menstrual cycle phase, OC use, or even sex.
Interestingly, although prior work has shown sex differences in favor of women on verbal learning and memory tasks, sex differences did not occur in Study 2 for the HVLT and verbal fluency. Prior work has shown that women perform better than men on verbal recollection tasks such as the CVLT, with no sex difference in verbal recognition (Kramer et al., 1988). Study 2 replicated results for verbal recognition, given that there was no sex difference, but not for verbal recollection. However, the two verbal recollection tasks do have some differences. First, there are more words listed in the CVLT and five trials are administered. Additionally, a distractor list is presented before the final recall. The HVLT is brief and only has three learning trials. Differences between the tasks could have impacted learning and memory for the presented words. For example, as discussed higher memory loads can negatively impact behavior, compared to lower memory loads (de Fockert et al., 2001; Joseph et al., 2012; Rypma & D’Esposito, 1999). The difference in memory load could have impact why studies see sex differences on the CVLT and why we did not see sex differences on the HVLT. Furthermore, Kramer and colleagues (1988) also assessed strategy use. They found that women tended to use semantic methods of grouping words, whereas men used serial order to remember words in the order they were presented. Study 2 did not measure strategy use. It is possible that there were differences in strategy between Study 2 and the Kramer et al. (1988) study would may have led to differences in behavioral performance.

Although, researchers have found that women had better recollection during the three trials of the HVLT and better word discrimination during the recognition trial, compared to men (Munro et al., 2012), it is important to note the age ranges of the participants. Participants in Study 2 ranged from 18 to 25 years old. The ages of participants in the Munro et al. (2012) study ranged from 67 to 89 years old, although none were diagnosed with any form of dementia.
Research has shown that cognitive decline can occur as early as middle aged to the onset of 60s, depending on the type of cognitive ability (Deary et al., 2009). It is possible that the sex difference that occurred in the Munro et al. (2012) study may be related to differences in cognitive decline, rather than differences in verbal recollection and recognition behavior. One study examined the differences in verbal abilities between young adults (aged 18 to 28) and non-demented older adults (aged 63 to 88) on vocabulary, reading ability, and verbal fluency (Kemper & Sumner, 2001). Results indicated that older adults had significantly greater vocabulary scores, than younger adults. However, older adults had significantly slower reading rate, reduced reading comprehension, and lower verbal fluency, than young adults. The researchers suggested that the older adults had decreased processing efficiency and lexical flexibility based on structural modeling analyses. Additionally, they found that working memory was heavily associated with reading skills, which was less efficient for older adults (Kemper & Sumner, 2001). Others have assessed how attention plays a key role in verbal memory tasks. In one study, researchers showed young adults (aged 21 to 31) and older adults (aged 63 to 76) multiple word pairs during a PET scan (Anderson et al., 2000). Participants were to remember the pair for later recall, immediately and after a delay, during which one word was presented and participants were to identify the previously paired word. The immediate recall was considered the encoding phase and the delayed recall was the retrieval phase. A secondary task was also performed at both phases. For a full attention condition, participants had to press a button every time a tone was played. For a divided attention condition, participants had to press different buttons, based on the tone of the sound, thus requiring additional attentional processes. Results indicated that young adults had significantly greater words recalled than older adults at both encoding and retrieval under both attention conditions. Additionally, both young and older adults
had significantly lower encoding scores during the divided attention condition, than the full attention condition. However, young adults had no difference in retrieval between the two conditions. In contrast, older adults had lower retrieval scores for the divided attention condition, compared to the full attention condition. The researchers also observed different patterns of activation in the prefrontal cortex between young and older adults at encoding and retrieval for both attention conditions, suggesting that differences in memory performance may be related to differences in prefrontal cortex activation (Anderson et al., 2000). Overall, the findings from this study suggest that older adults were more negatively impacted by the divided attention condition, than the younger adults. Related to cognition in aging adults, researchers also assessed how estrogen replacement therapy affected verbal memory in post-menopausal women over the age of 55 years (Kampen & Sherwin, 1994). Results indicated that women on estrogen replacement had significantly greater scores on recollection of paragraph details and a list of 12 words at both immediate and delayed recall, compared to women not on treatment. Although only young adults were tested in Study 2, future work can compare verbal memory across different groups of ages to assess any impact of cognitive decline and HRT in post-menopausal women.

The findings from Study 2 revealed no sex differences in verbal fluency as well. This was an unexpected finding given prior work that has shown that women perform better than men on verbal fluency tasks (Lewin et al., 2001; Munro et al., 2012; Weiss et al., 2003; 2006). The task was administered as the protocols described in other work. Others have attempted to provide normative data for performance on the verbal fluency task (Tombaugh et al., 1999). Researchers found no sex differences in performance with participants ranged from 16 to 95 years of age. The average score for men ($M = 37.0, SD = 13.0$) and the average score for women ($M = 37.8, SD = 13.1$) were comparable (Tombaugh et al., 1999). In Study 2, the average score for men ($M = 37.0$) and the average score for women ($M = 37.8$) were comparable (Tombaugh et al., 1999).
40.72, $SD = 8.48$) and women ($M = 40.32$, $SD = 8.14$) were also similar. Furthermore, the scores observed in Study 2 were in line with the scores found in the Tombaugh et al. (1999) according to the similar age ranges. They found that scores for participants between the ages of 16 and 19 years old ($M = 39.3$, $SD = 12.0$) were similar to participants between the ages of 20 to 20 years old ($M = 41.2$, $SD = 9.2$). Unfortunately, they did not report sex differences within the specific age ranges (Tombaugh et al., 1999). Regardless, the results from Study 2 and the results from prior work (Tombaugh et al., 1999) has shown no sex differences in verbal fluency performance.

Other work has examined how task instruction and strategy use can affect sex differences in verbal fluency. In one study, women in the luteal phase of their cycle and men performed a verbal fluency task with three different conditions (Scheuringer et al., 2017). Under a neutral condition, the task was given as typically presented (as was in Study 2), with no further instructions. Under a clustering condition, participants were to produce consecutive words that were phonemically similar (i.e. words that rhyme, differ by only one letter, etc.). Under a switching condition, participants were to produce consecutive words that were phonemically dissimilar. No sex differences were observed in the number of words produced during any of the conditions. However, women were more likely to use a switching method during the neutral condition, without being instructed to use the strategy. Researchers suggest that sex differences likely do not occur in verbal fluency itself. However, they believe that perhaps men and women employ different strategies to complete the task (Scheuringer et al., 2017). Task instructions may affect the type of strategy utilized by participants. The task administered in Study 2 was comparable to the neutral condition of this study, thus the lack of sex difference was replicated. However, it is possible that prior work showing sex differences on this task had provided different instructions that could have affected participants’ strategies. Future work should
examine strategy use in participants. Additionally, women in the Scheuringer and colleagues (2017) study were in the luteal phase of their menstrual cycle. During this phase, estradiol levels are medium to high and fall towards the end of this phase. However, progesterone levels are also elevated during the luteal phase. Studies should examine how verbal fluency and strategy use is affected by menstrual cycle phase in women.

5.1.7 Oral Contraceptives. As mentioned, OCs inhibit ovulation by increased exposure to exogenous ethinylestradiol and progestin which subsequently decreases endogenous estradiol and progesterone (Mordecai et al., 2008). With extended OC use, endogenous production of estradiol and progesterone can decrease to levels that are analogous with the follicular phase in nonusers (Mishell et al., 1972). Some types of OCs have active and inactive phases (van Heusden & Fauser, 2002; Mordecai et al., 2008). Active phases would include taking the exogenous hormones, whereas inactive phases would include taking a placebo. This allows the hypothalamic-pituitary-gonadal axis to slowly regain normal activity during the inactive phases (van Heusden & Fauser, 2002). The types of OCs that have these inactive and active phases are known as biphasic or triphasic OCs. The three current studies attempted to control for any differences in OC type by including participants on select types of OCs. Specifically, those on monophasic OCs with a small range of ethinylestradiol and progestin were recruited and included in analyses.

In assessing the impact of OC in women across multiple behavioral tasks, the differences between OC users and non-users were unexpectedly minimal. It was predicted that with a lowered exogenous levels of a synthetic estradiol hormone, women on OCs would have impaired performance in spatial memory. Overall, the OC group performed better than the LE group in probe trial performance at the start of day two on the VWMT in Study 1. The OC group also had
more improvement in object location performance, evidenced by lower difference scores, compared to men in Study 3. However, on the SWMT in Study 3, even though men had impaired performance to the ovulatory and non-ovulatory groups, men did not differ in performance compared to the OC group. Finally, men did perform better than the OC group on the VWMT in Study 3, as the expected sex difference. The findings from all three studies suggest that OC use did not induce vast impairments in learning and memory. This finding is important for the large population of women who use oral contraceptives. OC users can be more confident that use of an OC does not necessarily lead to cognitive impairments, at least with spatial memory. However, what remains unknown is if there are any long-term effects of OC use across an extended period of time. The participants used in these studies were college-aged undergraduate students who were typically only on an OC for a short amount of time (Study 1 – $M$: 2.33 years, $SD$: 1.65; Study 2 – $M$: 2.28 years, $SD$: 1.69; Study 3 – $M$: 2.11 years, $SD$: 1.39). Future research should examine how OC use impact not only spatial memory performance, but other cognitive abilities as well.

Furthermore, the impact that OC use has on the developing brain, for women who start at an early age is still debated. OCs have been prescribed for uses other than birth control as well. OCs can be taken to treat acne, seborrhea, menstrual cramps, and menstrual regulation (Huber & Walch, 2006). Additionally, due to the other applications of OCs, they are commonly prescribed to young adolescents, starting around the age of 14 years old (Huber & Walch, 2006). In one study, structural images were obtained from healthy young adults (aged 20 to 30) to assess sex differences, effects of menstrual cycle phase, and impact of OC use (Pletzer et al., 2010). Results indicated that men had larger overall gray matter volume compared to all groups of women. Additionally, naturally cycling women in the follicular or luteal phase had no differences with
each other. However, the naturally cycling women had significantly greater gray matter volume than women on OCs. In examining specific regions within the brain, it was shown that OC women had significantly larger prefrontal cortices, pre- and post-central gyri, parahippocampal and fusiform gyri, and temporal regions, than both groups of naturally cycling women (Pletzer et al., 2010). Another study examined brain volume in women who had never used an OC, women who continuously used one type of OC, and women who used multiple OCs (Pletzer et al., 2019). None of the women were users at the time of the study. There were no differences in gray matter volume between the three groups of women. Additional analyses revealed that duration of previous OC use was positively correlated to hippocampal and basal ganglia volumes, bilaterally. In controlling for the amount of time since discontinuation of the OC, the correlation for the hippocampus was no longer apparent. The researchers suggest that perhaps the effects of OC use on the hippocampus are potentially reversible after OC use is discontinued (Pletzer et al., 2019). Overall, the findings from these studies suggest that OC use does have structural impacts on the brain, however it is unclear whether those structural changes result in behavioral differences and if the changes are prevalent over time.

5.1.8 Progesterone. While estradiol did have some implications on behavior, it is also important to consider the relationship that progesterone has with estradiol and the potential impact on behavior. Across a menstrual cycle, progesterone levels are low during the follicular phase and high during the mid-luteal phase. Salivary assays were obtained from participants in Study 1 to quantify level of estradiol. However, due to financial restrictions, assays of progesterone were unable to be obtained. It should be noted that progesterone could have mediated the results of Study 1. Specifically, it was found that the high estradiol group had better memory retention at the start of day two on the VWMT, than the low estradiol group. Prior
literature would suggest that progesterone may have been involved in either enhancing performance of the HE group or impairing performance of the LE group. As mentioned, progesterone enhanced the effects of estradiol on hippocampal structure (Woolley & McEwen, 1993). These researchers observed increases in dendritic spine densities during the proestrus phase of a female rat’s cycle, compared to the estrus phase. It was observed that progesterone initially aids estradiol in increasing spine density during proestrus and then subsequently is involved in dramatically lowering dendritic spine densities when the estrus phase is reached (Woolley & McEwen, 1993). In another study, OVX rats with an adrenalectomy were treated with either estradiol alone or estradiol with progesterone and tested behavioral performance in a spatial water maze (Snihur et al., 2008). Results showed that in the absence of adrenal steroids, OVX rats treated with estradiol had impaired performance on the task compared to control rats and OVX rats treated with estradiol and progesterone. These findings suggest that the beneficial effects of estradiol treatment in OVX rats depends on either the presence of adrenal steroids or progesterone (Snihur et al., 2008). The overall results from these studies imply that progesterone plays an important role in facilitating estradiol to complete its’ actions properly. Thus, future studies should measure both progesterone and estradiol levels from participants to account for any overlap in function.

Others have also found that progesterone alone, progesterone in combination with estradiol, or estradiol alone was able to reverse the behavioral impairment induced by scopolamine, a cholinergic antagonist, on radial arm maze performance in OVX rats (Tanabe et al., 2004). However, progesterone treatment alone was not able to impact spatial memory performance prior to scopolamine treatment, compared to the OVX untreated rats, even though both estradiol treatment alone and estradiol combined with progesterone treatment was able to
(Tanabe et al., 2004). Thus, it seems that progesterone can aid estradiol in its impact on cognition but that it may be limited on its own.

Participants in Studies 2 and 3 were categorized into hormone groups based on menstrual cycle information. With the start of menstruation characterized as day 0, those that were between days 0 to 8 or within days 25 to 35 were considered to be in the non-ovulatory phase. These women would have low estradiol levels given that the day ranges include those in the late-luteal and early follicular phases. These women would also have low progesterone levels. Those that were between days 10 to 17 were considered to be in the ovulatory phase. These women would have high estradiol levels which is indicative of the late-follicular and ovulatory phases. These women would also have low progesterone levels. Women between any of the remaining days were excluded due to likely have medium levels of estradiol and elevated levels of progesterone. Thus, many attempts were made to minimize the potential impact of progesterone.

Research has examined how progesterone impacts learning and memory in rodents (Gibbs, 2000). OVX female rats received hormone treatments at various time points following surgery for a total of 5 groups. One group received estradiol immediately following the surgery. Another group received estradiol 3 months post ovariectomy. Another group received both estradiol and progesterone 3 months post ovariectomy. One group received both estradiol and progesterone 10 months post ovariectomy. Finally, the control group was OVX female rats that received no hormonal treatment. Female rats were assessed on a delayed matching-to-position spatial memory task (paired with a T-maze) 8 to 12 months after the ovariectomy. Control rats that received no hormonal treatment required more days to reach criterion than all other groups. Rats that received both estradiol and progesterone 3 months post-surgery required the least number of days to reach criterion. All groups treated with hormones improved at a faster rate on
the task than the control group. Overall performance was the best for the group that received both estradiol and progesterone 3 months post-surgery (Gibbs, 2000). The findings from this study emphasize the important role that progesterone had in enhancing the beneficial effects of estradiol. When rats received both hormones, they performed better than rats that received only estradiol. Another study assessed the impact of progesterone treatment on object and spatial memory (Harburger et al., 2008). Young OVX mice trained on an object recognition task and the Morris water maze were tested for retention after 24- or 48-hour delays. Mice received an injection of 5 mg/kg, 10 mg/kg, 20 mg/kg, or a vehicle following training of each task. Results indicated that mice that received either the 10 or 20 mg/kg dose after training on the object recognition task had enhanced memory compared to the vehicle treated group. However, any treatment of progesterone had no impact on spatial memory (Harburger et al., 2008). These results suggest that the impact of progesterone on memory may be limited to some types of learning and that a large enough dose needs to be administered to have an impact.

Progesterone is also often used in conjunction with estrogen in HRT (Maki et al., 2001; Sherwin, 2007). Some work has shown no differences between HRT with estrogen alone or with estrogen plus progesterone. Results from one study showed that women with either hormonal treatment had fewer working memory errors and faster completion times compared to the control women, on a spatial working memory task (Duff & Hampson, 2000). Other work has shown enhanced verbal memory on the CVLT in post-menopausal women taking HRT either with estrogen alone or in conjunction with progesterone, compared to no treatment (Maki et al., 2001; Resnick & Maki, 2001).

5.2 No Effect of Anxiety on Behavior
To assess how general anxiety impacts spatial and verbal memory, participants in Study 2 filled out measures of anxiety and mood and completed the VMWT, HVLT, and verbal fluency task. Participants were categorized as anxious if they scored high on the STAI which measures for both state and trait anxiety. The STAI measures subjective feelings of nervousness, worry, apprehension, calmness, confidence, security, and activation of the autonomic nervous system (Julian, 2011; Spielberger et al., 1983). As discussed, trait anxiety refers to feelings of apprehension, tension, and worry, whereas state anxiety is an emotion state that fluctuates in response to stressors (Spielberger, 2013). It is important to note that participants self-reported subjective ratings on the STAI. Disturbances in attention, motivation, or understanding the survey could have affected the categorization on participants. Furthermore, individuals with a current or previous diagnosis of a stress, anxiety, or depressive disorder were ineligible for the study. However, since this was also based on self-report, it is possible that an individual with a diagnosis could have participated in the study.

In examining the effects of general anxiety on spatial navigation, the results from Study 2 showed no group differences between the anxious and non-anxious groups on distance to platform or probe trial performance. Anxiety did not interact with sex or groups of women. This suggests that general anxiety does not impact performance on the VMWT. However, the effects of anxiety on long-term retention were not examined. The second part of the VMWT was not administered. Future studies should aim to investigate long-term effects of anxiety on spatial navigation. In assessing the impact of anxiety on verbal memory, results from Study 2 showed no difference between the anxious and non-anxious groups in addition to no difference between sexes or groups of women. These findings further suggest that general anxiety may not be a strong enough factor to influence behavior. Based on the results from Study 2 it is apparent that
scoring high on the STAI did not impair performance on any of the behavioral tasks, as predicted. It is possible that if individuals have had elevated anxiety for an extended period of time that they have learned to cope with it. Additionally, information about the causes of anxiety, history with anxiety, length of time dealing with anxiety, and coping mechanisms to manage anxiety was not obtained. Future studies should consider obtaining this information to further understand the impact that anxiety has on daily life.

Prior work has shown impaired memory due to high anxiety (Bowman et al., 2019). In one study, researchers assessed the impact of anxiety on a prospective memory task (memory for a task to be completed in the future). Participants were given instructions for a prospective memory event to be completed during three ongoing cognitive tasks. Those that scored high on the STAI had poorer memory performance than those with low anxiety scores. Additionally, high anxiety on the STAI was positively correlated with negative affect, measured by the PANAS (Bowman et al., 2019). In another study, researchers examined how induced anxiety impacted memory (Hallford et al., 2019). Participants in the anxiety-induced condition viewed a series of anxiety-provoking statements while listening to an anxiety invoking piece of music. Results indicated that the anxiety-induced group had significantly lower scores on an autobiographical memory task, compared to the neutral group that was exposed to neutral stimuli. However, induced anxiety had no effect on verbal fluency and working memory, assessed by a digit span test (Hallford et al., 2019). Overall, the findings of these studies indicate that anxiety may impact some types of memory but the impact on different types of memory are inconsistent.

Although anxiety did not impact behavior in Study 2, it was related to mood. Results indicated that non-anxious participants had higher positive affect on the PANAS than anxious
participants. Anxious participants had higher negative affect on the PANAS than non-anxious participants. Given that anxiety encompasses negative feelings of apprehension, tension, and worrying along with activation of the autonomic nervous system (Spielberger, 2013), it is not surprising that those with higher levels of anxiety express more negative mood and those with lower levels of anxiety express more positive mood. Related to the PANAS, results from the POMS revealed that the anxious group showed higher scores for tension, depression, anger, vigor, fatigue, and confusion than the non-anxious group. Again, based on the feelings associated with anxiety, it is justifiable that individuals with higher levels of anxiety would exhibit moods that are more negative in nature. There were no correlations with mood scales and behavioral performance on any of the tasks. Furthermore, the findings from this study demonstrate that having elevated levels of anxiety are associated with more negative moods and feelings which may further perpetuate the anxiety itself. Additionally, high anxiety was negatively correlated with positive affect, indicating that these individuals likely have fewer positive feelings and moods but that this was unrelated to behavioral performance on spatial and verbal memory tasks.

5.3 The Impact of Stress on Spatial Memory

To investigate the impact of stress on spatial memory, behavioral performance was assessed on the VMWT, object location task, SWMT, and the MRT in Study 3. Acute stress was induced by use of the vTSST. Participants were told that they would orally give a speech to two faculty members in a virtual environment. In actuality, no faculty members were involved in the task. The virtual characters posing as faculty members were run by an experimenter in another room. To assess the validity of the stressor, physiological and subjective stress was measured. Elevations in heart rate were monitored throughout the task and were compared to heart rates during baseline (at the start of the study). Subjective ratings of stress were given four times
throughout the experimental session, once at the start of the study, once before the stress or control task, once immediately after the stress or control task, and once at the end of the study. Results indicated that participants had significant elevations in heart rate throughout the stress task compared to participants in the control task. Stressed participants also had significantly higher subjective ratings immediately following the task and at the end of the study compared to control participants. The physiological and subjective measures positively correlated with each other indicating that as heart rate increased, subjective stress ratings increased as well.

5.3.1 Spatial Memory. To assess spatial navigation, participants completed the VMWT prior to and after the induction of stress to measure the change in performance. As previously discussed, prior to the stressor, men had better performance than women in distance to find the platform and probe trial performance. During the post-test session, there were no sex differences on distance to platform. However, men performed better than women in probe trial performance at the start and end of this session. There was also an effect of stress on probe trial performance for the probe at the start of the post-test, in that both stressed men and women performed better than control men and women. This suggests that induction of an acute stressor enhanced performance for both men and women compared to their control counterparts.

Additionally, in examining the change in performance from pre-test to post-test, a difference score was calculated between the percentages of distance in the correct quadrant from the probe trial at the end of the pre-test and the probe trial at the start of the post-test. Results showed that control groups had greater differences scores, indicating that they had a large reduction in percent of distance in the target quadrant from pre-test to post-test, compared to the stressed groups. The findings from this study indicating that men also benefitted from acute stress were unexpected. However, some research has shown that men have greater responses to
cortisol (Andreano & Cahill, 2006; Guenzel, et al., 2014; Mazurka et al., 2017; Zorawski et al., 2006) which can lead to enhanced learning and memory (Andreano & Cahill, 2006; Zorawski et al., 2006). However, for women, research has shown that elevations in cortisol may not be related to behavioral performance (Andreano & Cahill, 2006; Zorawski et al., 2006). Others have shown that elevations in cortisol are imperative for impacting behavior regardless of sex (Buchanan & Tranel 2008). These researchers found that those who had high increases in cortisol due to a psychosocial stressor had impaired emotional memory. In contrast, participants who did not have elevations in cortisol from the stressor had enhanced performance for some emotional stimuli (Buchanan & Tranel 2008). Thus, it seems that feeling the physiological effects of stress is important to influencing behavior. While Study 3 did not measure levels of cortisol, having that information may be valuable in understanding how men and women respond to stress and how that response affects behavior. Overall, the findings from induction of stress on spatial navigation performance indicate that both stressed men and women were protected from reductions in memory from pre-test to post-test compared to control men and women. This suggests that an acute stressor may benefit learning and memory on a task like the VMWT.

As discussed, women had better performance on the post-test trial of the object location task, compared to men. However, there was no effect of stress on overall score on that trial. Based on a difference score in calculating the change in performance from pre-test to post-test, results indicated that women were unaffected by the induction of stress and performed just as well as controls on the post-test trial of this task. Men who were exposed to the stressor performed significantly worse compared to control men on the post-test trial of this task. Given that this task is typically female dominated, it is interesting to see that an acute stressor was unable to hinder performance in women. Perhaps this occurred because women already
accelerated on this task. Control men performed worse than stress and control women demonstrating that even without exposure to the stressor, men had impaired performance. Therefore, with added acute stress, it is not surprising that stressed men performed the worse overall. These findings once again suggest that object location is a different type of spatial memory than tested in the other tasks. Furthermore, the hypotheses that women would be unaffected by induction of stress, whereas men would have hindered performance by induction of stress, were supported for this task.

Prior work has also shown a sex difference in favor of women (Honda & Nihei, 2009; Levy et al., 2005; Spiers et al., 2008). However, it seems that an acute stressor heightened the sex difference in Study 3, given that stressed men were even more impaired on the task than control men. Men have been shown to have a greater cortisol response to stress (Andreano & Cahill, 2006; Guenzel et al., 2004; Mazurka et al., 2017). However, in some cases greater elevations in cortisol have led to memory impairments (Espin et al., 2013; Kirshbaum et al., 1996) and in other cases memory enhancements (Andreano & Cahill, 2006; Zorawski et al., 2006). Rodent work has observed impairments in males in response to stress on many different tasks including object location (Beck & Luine, 2002), the radial arm maze (Luine et al., 1994; 1996), a Y-maze (Conrad et al., 2003), and the Morris water maze (Kitraki et al., 2004).

However, the type of stress used these experiments were chronic restraint stress that lasted for 21 days. In a series of experiments, Lupien and colleagues (1994; 1995; 1998; 2002) examined cortisol levels, memory, and hippocampal volume in a group of healthy older adults (aged 60 to 80 years) for three to six years in a longitudinal study. One group of individuals (37.9%) had significant increases in high cortisol level across the span of the study (Lupien et al., 1995). Another group (46.7%) had moderate increases in cortisol level and the final group (15.3%) had
moderate decreases in cortisol level (Lupien et al., 1995). In another study, the researchers observed that the group with high cortisol had impaired explicit memory on a cued recall test and selective attention on a visual search task, compared to both other groups (Lupien et al., 1994). Furthermore, the group with decreased cortisol performed as well as healthy young adults (aged 22 to 24 years) on cognitive tasks assessing explicit and implicit memory, attention and verbal fluency (Lupien et al., 1994). In a follow-up experiment, it was observed that participants with significant increases in cortisol had hippocampal volumes that were 14% smaller than the group with moderate increases (Lupien et al., 1998). Additionally, the group with decreased cortisol had hippocampal volumes that were 14% larger than the group with moderate increases (Lupien et al., 1998). Finally, to assess whether memory impairment in the high cortisol group was related to the high circulating levels of cortisol or to long-term exposure to glucocorticoids, researchers inhibited cortisol secretion by administrating metyrapone and then restored baseline cortisol levels by infusing hydrocortisone (Lupien et al., 2002). Declarative memory was examined on a free recall test of a list of words across multiple trials. Results indicated that for the high cortisol group, metyrapone had no impact on memory. However, hydrocortisone significantly impaired memory. For the group with moderately increased cortisol, metyrapone treatment impaired memory. This deficit was reversed with hydrocortisone replacement (Lupien et al., 2002). The findings of this study suggest that memory can be modulated by manipulations of glucocorticoids, however the direction of impact is reliant on cortisol history. Furthermore, these studies suggest that having an optimal level of cortisol is beneficial for memory. When cortisol levels are too high, there are impairments in memory and reductions in hippocampal volume. When cortisol levels are too low, there are also impacts on hippocampal volume. The hippocampal increase seen in the decreased cortisol group was not indicative of what was seen in
normal controls. Thus, it appears that having a balanced level of cortisol, which is released in response to stress, is optimal. Future work can attempt to identify the level of stress necessary to maintain an optimal level of cortisol.

Results from Study 3 also indicated that women performed better and had fewer working memory errors on spatial working memory task compared to men. However, there was no impact of stress induction on behavior as well. This suggests that induction of an acute stressor did not impact this spatial working task. Specifically, while it was expected that women may not be affected by the stressor, it was predicted that stressed men would have impaired performance compared to control men. The findings from the study did not support that hypothesis. Instead, stressed and control men did not differ in behavior. However, one study found working memory performance was impacted by stress (Lai et al 2014). In this study, participants underwent the cold-pressor task and the Trier social stress test. Results showed that participants in the stress group had enhanced working memory performance compared to the control group (Lai et al 2014). While this contradicts the results from Study 3, it should be noted that the working memory task was quite different, and two types of stressors were used. Lai and colleagues (2014) utilized both physiological and psychosocial stress.

Although there was no impact of stress on behavior in the SWMT in Study 3, there was a sex difference in favor of women. In contrast, men performed better on the MRT compared to women. However, there was no impact of stress on MRT performance as well. Participants in the stress and control conditions performed at the same level indicating that the vTSST did not impact mental rotation. These findings suggest that mental rotation ability may not be susceptible to the effects of an acute stressor whether it is impaired, as predicted for men, or enhanced, as predicted for women. Although the hypotheses were not supported for this task, the results of
this study demonstrate that different types of spatial memory, such as mental rotation, spatial navigation, object location, or spatial working memory, are not comparable and can lead to different results.

5.3.2 Induction of Stress. The stressor used in Study 3 was a virtual version of the Trier Social Stress Test developed by Fallon and colleagues (2016). In assessing the impact of the vTSST on cortisol levels, they showed that cortisol levels were elevated immediately following the stressor and remained elevated for up to 45 minutes post-stress. In examining subjective rating of stress, scores were the greatest immediately following the vTSST, replicated in Study 3, and then reached baseline values at 30 minutes post-stress (Fallon et al., 2016). No measures of subjective ratings were taken within those 30 minutes thus it is unknown at what exact point, the ratings started to decline. In using different versions of a virtual TSST, studies have found that elevations in cortisol in response to the stressor remained high and different from controls up to 40 minutes post-stress (Fich et al., 2014; Jonsson et al., 2010). Another study found that their version of the virtual TSST led to elevated heart rate, blood pressure, and cortisol level for 60 minutes post-stress induction (Hawn et al., 2015). Similar results have been found in the original non-virtual TSST in that cortisol response hits peak levels at 10 minutes post induction of stress, followed by reductions for the next 20 minutes (Kirschbaum et al., 1999; Zandara et al., 2016). All tasks in Study 3 were assessed within a timeframe during which the effects of stress would still be experienced. Thus, it seems as though the SWMT and MRT were unaffected by stress induction.

Other work has been done to compare different methods of stress induction, Giles and colleagues (2014) tested the effects of three methods on working memory (N-back test). The three stressors included the standard TSST, a socially evaluated cold pressor task (SECPT) in
which participants were videotaped for later assessment of facial expressions, and an arithmetic task (AT) in which participants had to solve math problems and received immediate feedback following each response to indicate if they were correct or not. Results indicated that both the TSST and SECPT induced elevated heart rate in comparison to the control group and to baseline. Elevated heart rate for the SECPT reduced after 10 minutes, whereas heart rate in response to the TSST remained elevated at the 30-minute check. Heart rate for the AT group did not differ across the session. Cortisol level was significantly elevated for the TSST and SECPT groups compared to controls and compared to baseline and remained elevated at the 20-minute check. The AT did not affect cortisol level. Interestingly, there were no differences in working memory performance between any of the groups (Giles et al., 2014). The findings of the study suggest that not all types of stressors elicit the same physiological reactions. Furthermore, a physiological reaction to stress does not always impact memory. The findings from Study 3 indicated that our participants experienced physiological and subjective stress in response to the vTSST, but that only spatial navigation and object location memory were affected by stress. There was no effect of stress on spatial working memory and mental rotation ability.

In another comparison study, researchers found that the TSST led to significantly greater increases in cortisol and adrenocorticotropin hormone (ACTH, another hormone that is released in response to stress) compared to the cold pressor task (McRae et al., 2006). However, this study found that there were no differences in subjective rating of stress in response to both stressors. Many believe that the TSST is more predictive of real-life stressful situations, given the psychosocial evaluation. Because of that, the socially evaluated CPT was designed in an attempt to combine a psychosocial and physiological stressor. In one study, researchers compared different presentations of the CPT (Schwabe et al., 2008). They found that the SECPT
had a significantly greater increase in cortisol level than the standard CPT. Both stressors had significant elevations in cortisol compared to the control, in which participants placed their hand in warm water. There were no differences between the SECPT and the CPT for elevations in heart rate, blood pressure, or subjective rating (Schwabe et al., 2008). Overall, it seems evident that having a psychosocial component to a stressor yields the greatest physiological and subjective response.

5.4 Summary

In reviewing the research done in the set of studies presented, it was shown that spatial memory is not only important to examine but is also susceptible to changes based on a variety of factors including estradiol level, menstrual cycle phase, sex, and induction of stress. In some cases, behavior was enhanced, in others behavior was impaired, and sometimes behavior was unaffected by these factors. Specifically, higher levels of circulating estradiol were shown to be beneficial in comparison to lower levels for memory retention in spatial navigation. Use of oral contraceptives did not lead to any behavioral impairments. Furthermore, general anxiety had no impact on behavior. However, it was observed that induction of external and unexpected acute stress can alter behavioral performance. Both men and women that underwent induction of stress had protected memory retention in spatial navigation compared to control participants. Induced stress also impaired performance on an object location task in men but had no impact in women. Stress did not affect all types of spatial memory tasks since there were no effects of the vTSST on spatial working memory and mental rotation ability. Thus, in researching spatial memory, it is important to consider how estradiol levels menstrual cycle phase, sex, and induction of stress impact behavior.
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